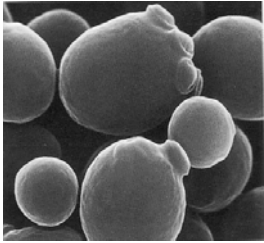


# Molecular Cell Biology of the Yeast *Saccharomyces cerevisiae*

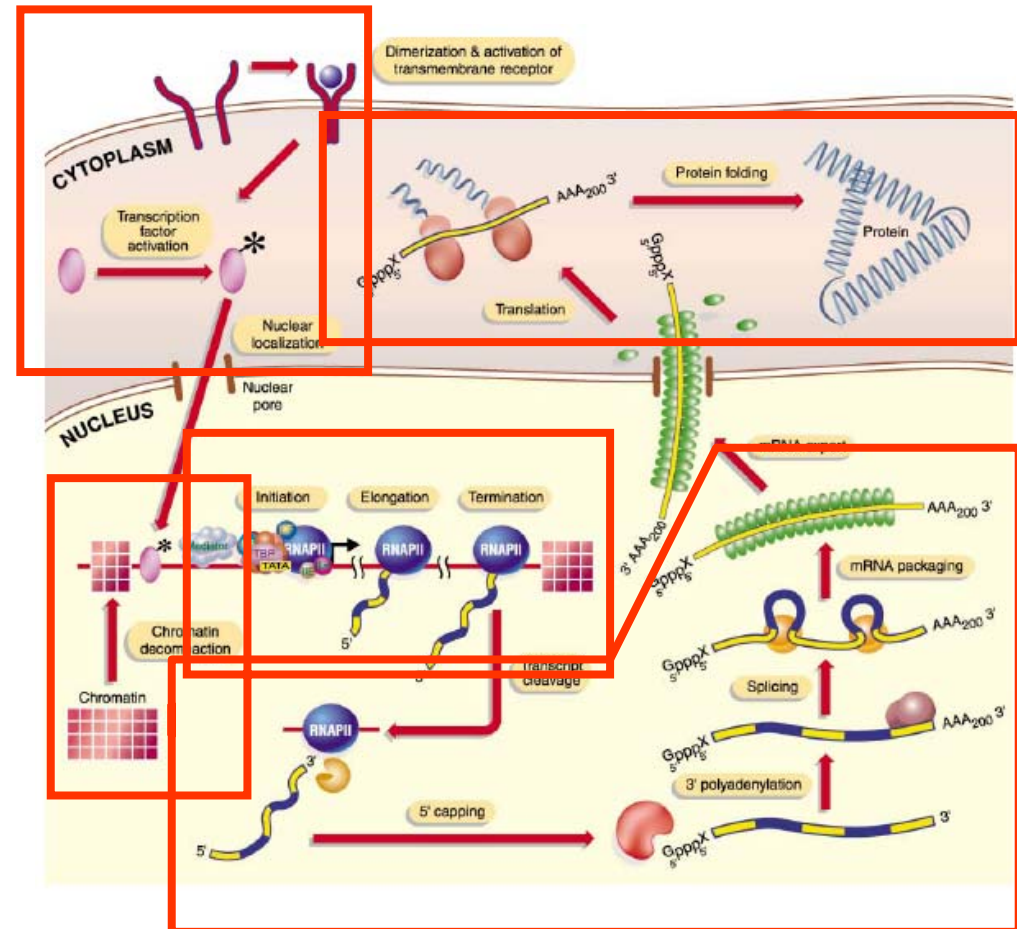
Lecture II: Transcriptional Regulation: Network Architecture,  
Molecular Mechanism, and Phenotypic Dynamics

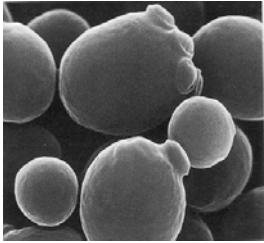
Zhang Yi,  
National Institute of Biological Sciences, 20080518



# Components of Gene Expression

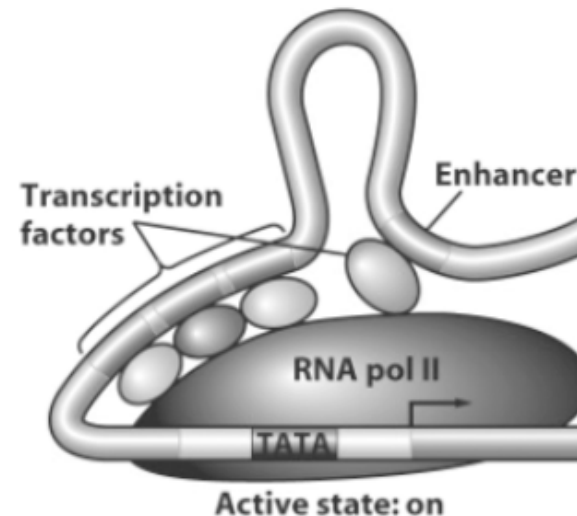
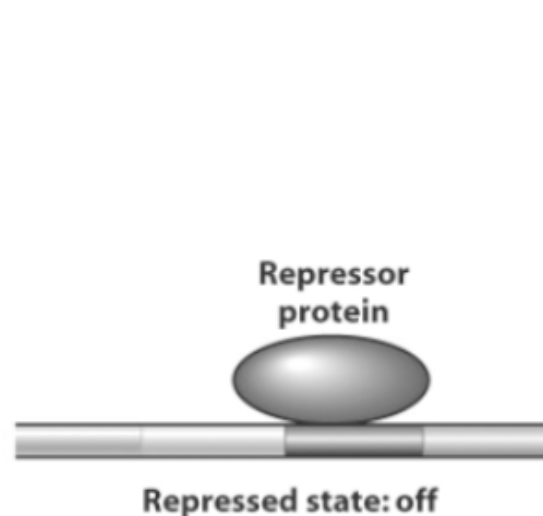
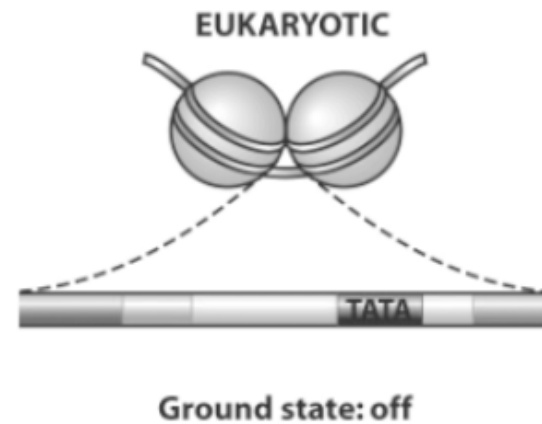
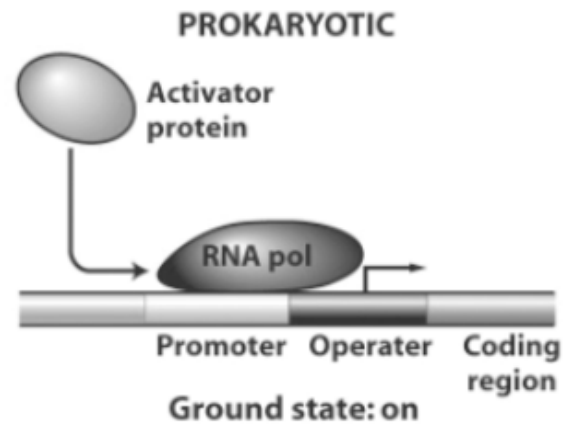
- Signalling Trigger
- Chromatin Dynamics
  - Histone Modification
  - Chromatin Remodelling
  - DNA editing
- Transcription Machinery Processing
  - Initiation
  - Elongation
  - Termination
- RNA Processing
  - Capping
  - Tailing
  - Splicing
  - Editing
  - Packaging
- Translational Regulation

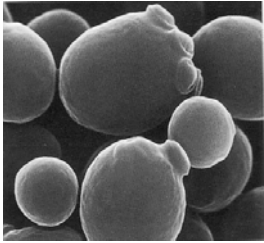




# Transcription Overview

- Default to ON for **prokaryotes** and OFF for **eukaryotes**.





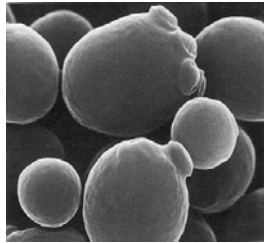
# Ptashne and transcription factors



Young...



Old (and stubborn enough ...)



# Gal4 Structure

Table 2. Internal Deletion Mutants

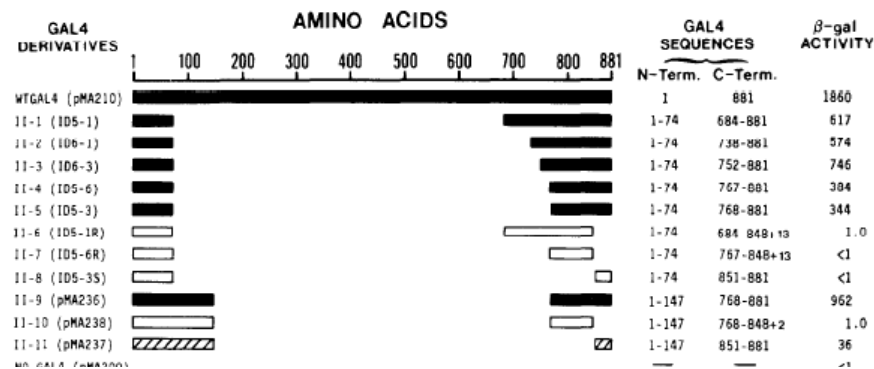
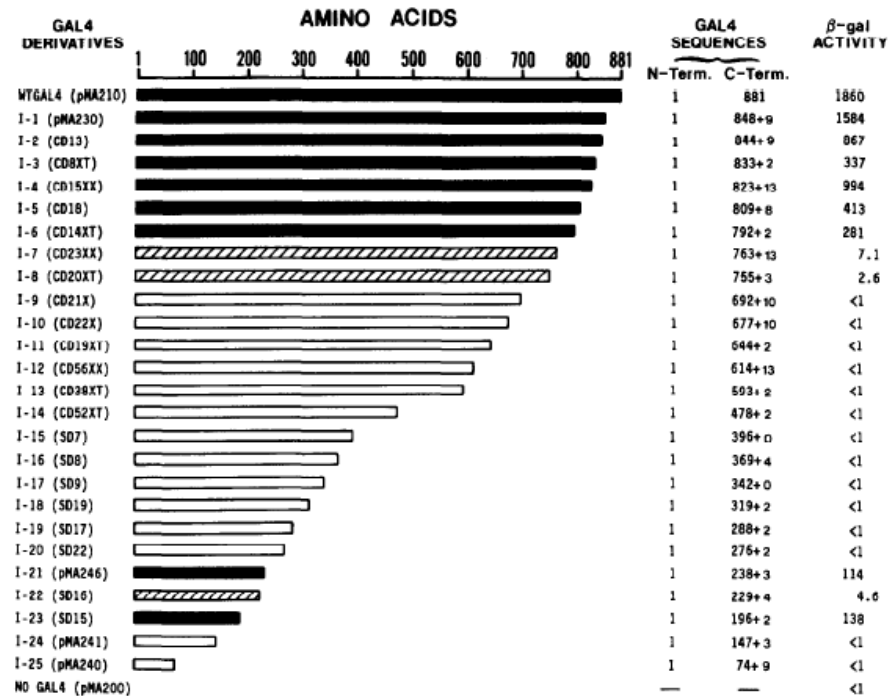
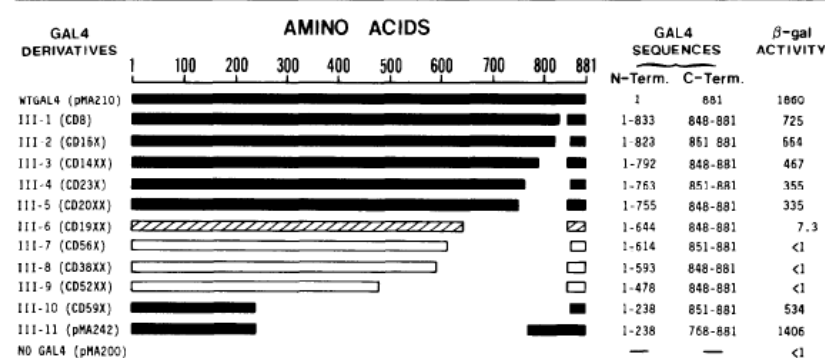
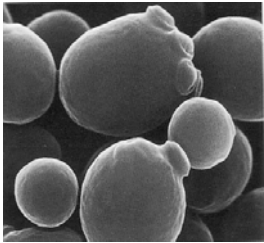
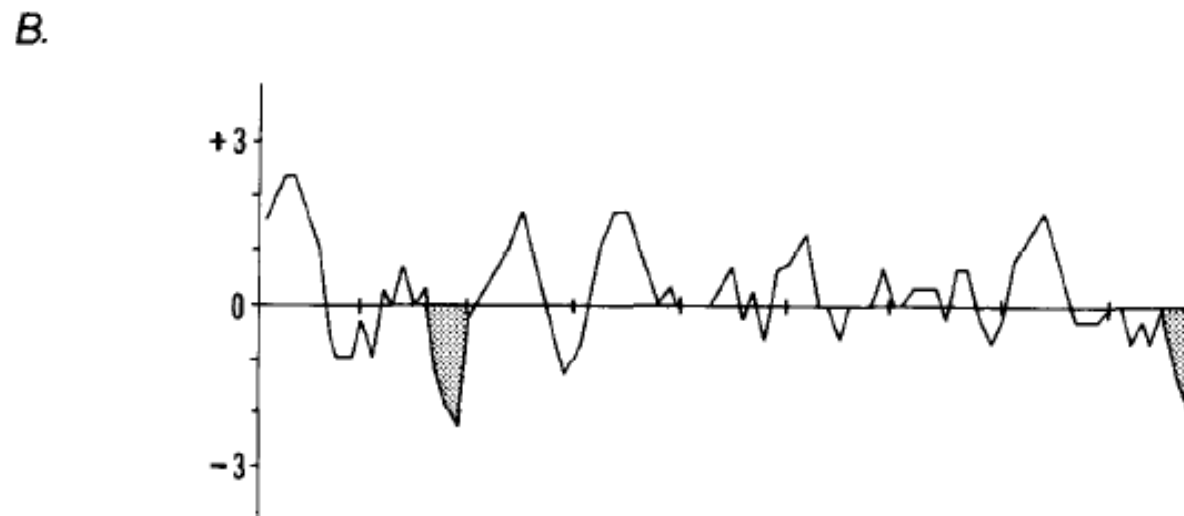
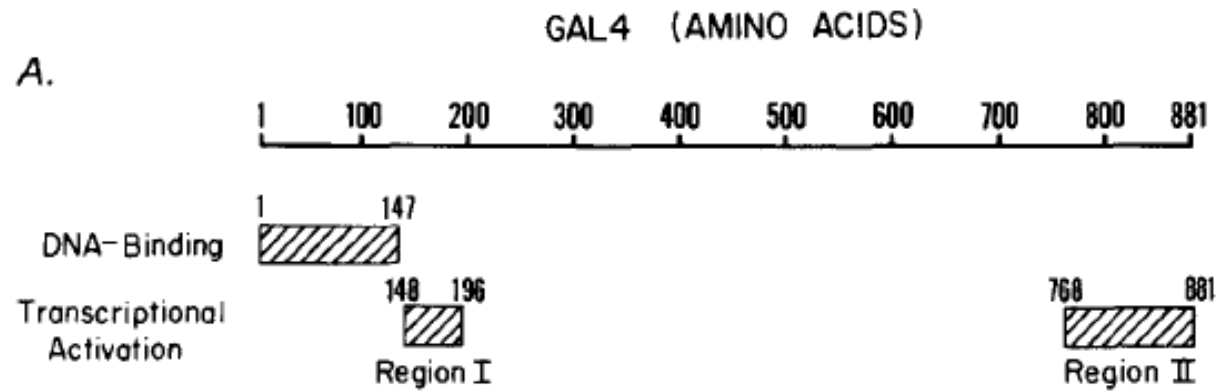


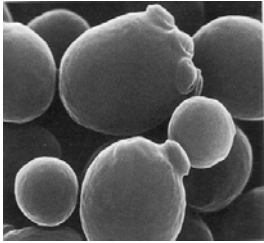
Table 3. Further Internal Deletion Mutants



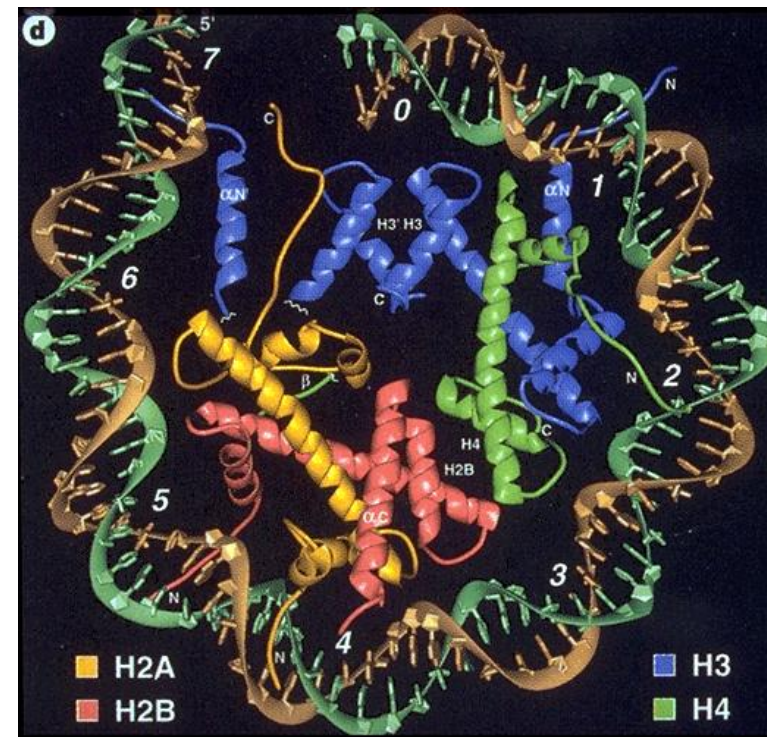
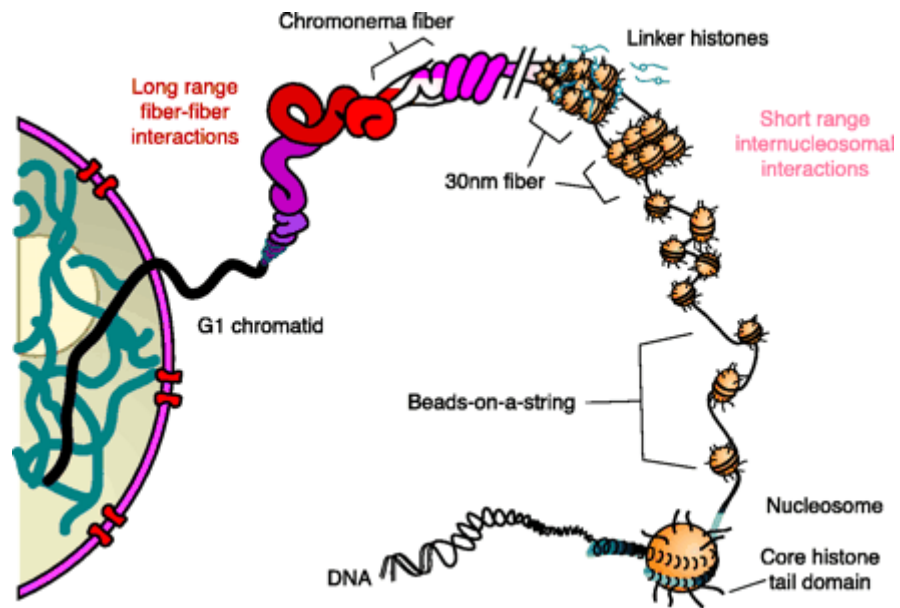


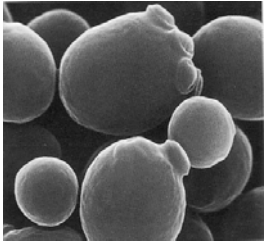
# Gal4 Structure



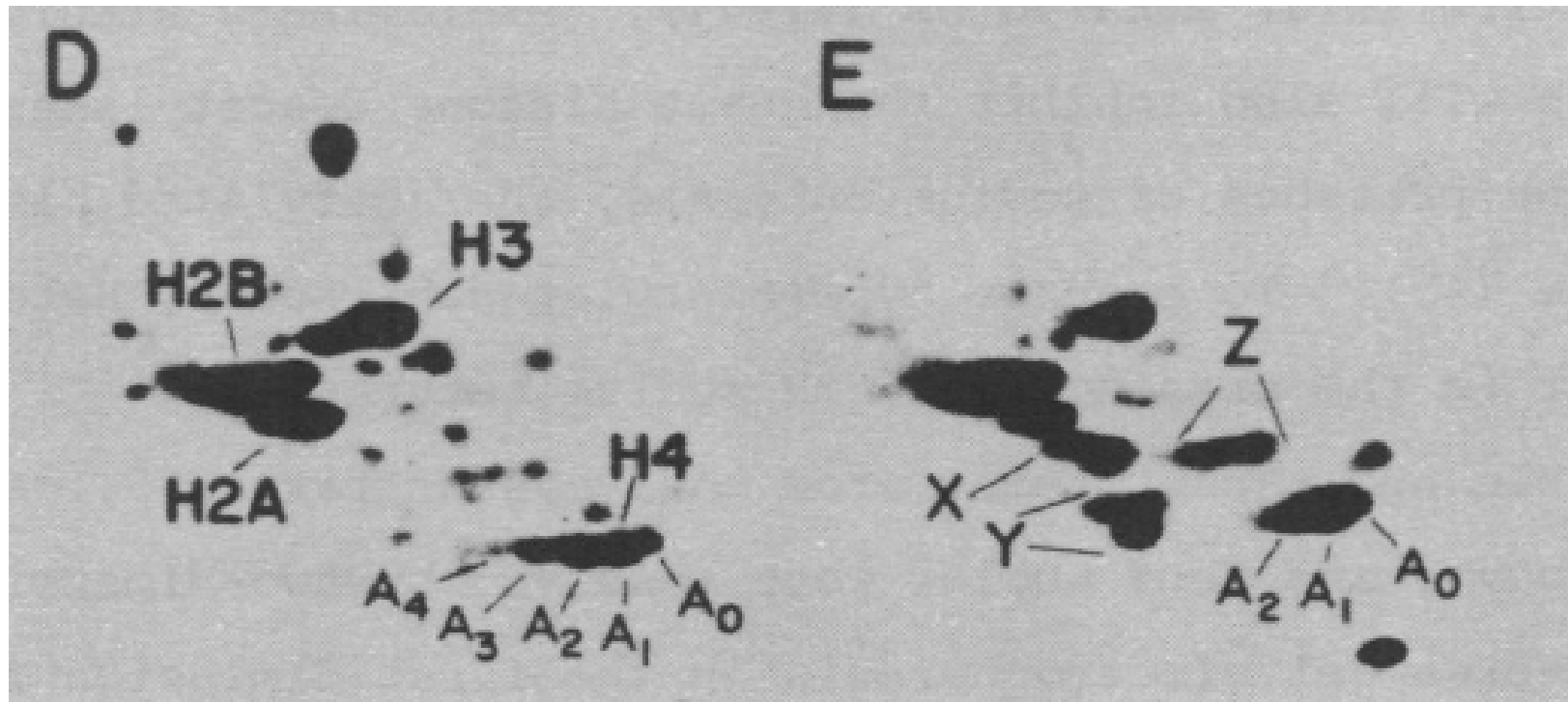


# Chromatin Structure



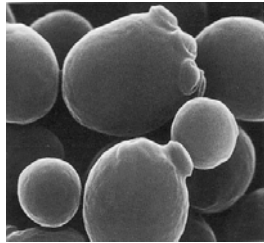


# Histone Acetylation



+100mM sodium butyrate in isolation





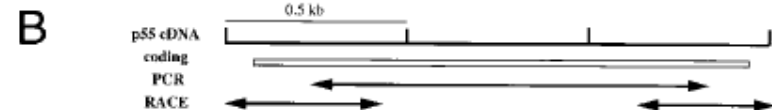
# Biochemical Cloning of a Tetrahymena HAT with homology to yeast Gcn5p

**Tetrahymena HAT A (p55)**

**A**

```

1  MADQEKSQAQDAQNAAPQETAFVGMNGEETGLGFATRDQGAKEEDQGLL 49
50  DFDILTNDGTHRNMKLLIDLKNIFSRQLPKMPKEYIVKLVLDRHHESMVI 99
100 LKNKQKVIGGICFRQYKQRFQFAEVAFLAVTANEQVRGYGTRLMNKPKDHM 149
150 QKQNIIEYLLTYADNFAIGYFKKQGFTKEHRMPQEKWKGYIKDYDGGTLME 199
200 CYIHPYVDYGNISQIIKQKELLIERIKKLSLNEKVFSGKEYAALIQNSM 249
250 DNEDPENPKVNPSPDIPGVAFSGWEWKDYHELKKSERSFNLCANVIGNM 299
300 KRHKQSWPFLDPVNDKDDVPDYDVITDPIIDIKAEKKLONNOYVDKDQFI 349
350 KDVKRIFTNAKIYNQPDITYYKAAKELEDFVEPYLTKLKDTPKESHTPSNN 399
400 NSAHGSKKPLPVSQKKVQKRNE 421
  
```

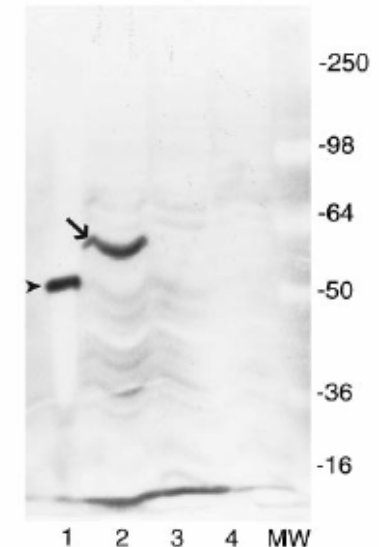


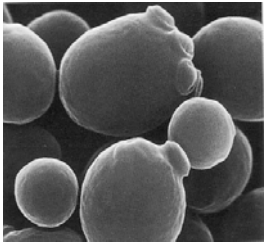
**Tetrahymena p55/yeast Gcn5p**

```

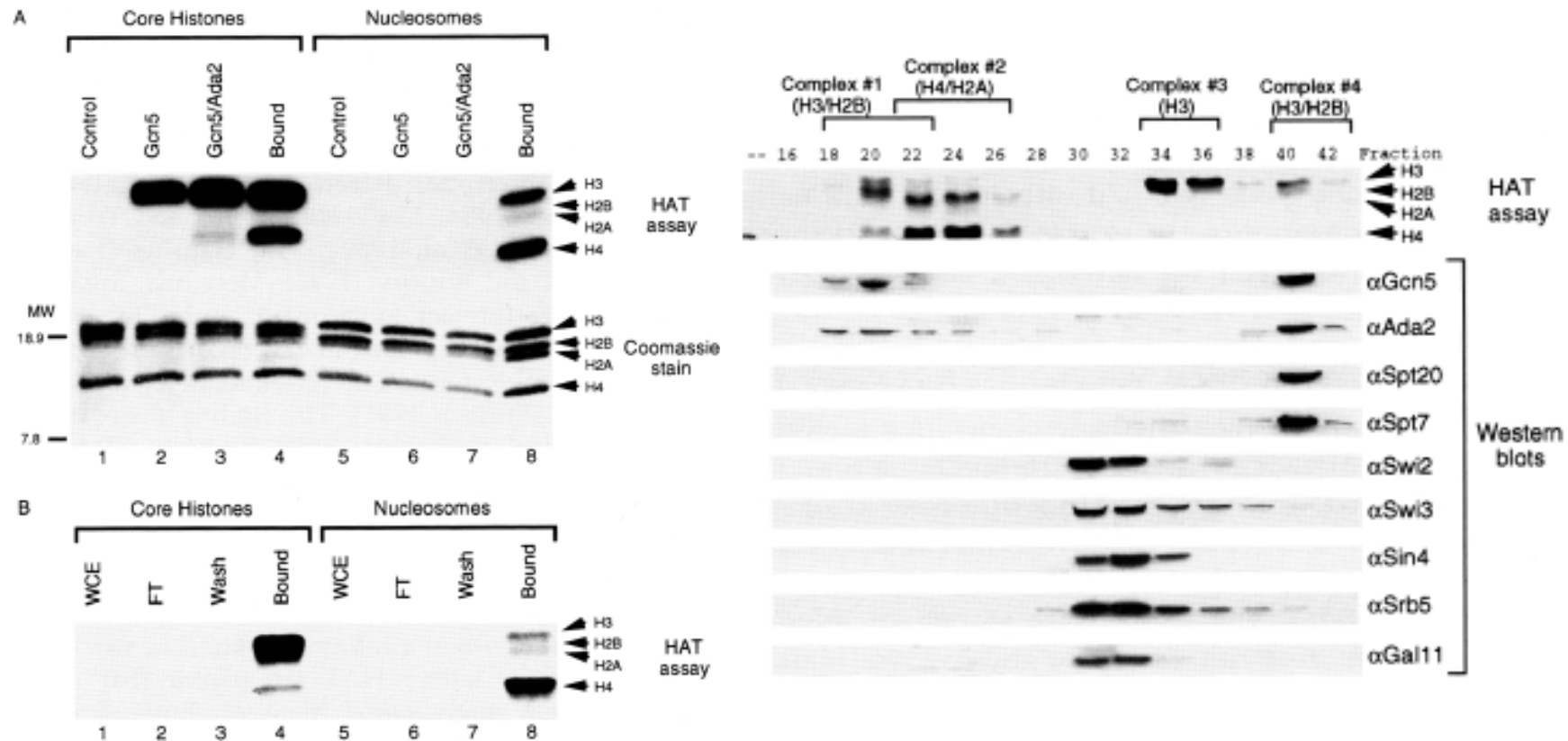
Gcn5p 1  MVTKHQIEEDHLGDATTDPVKKVLENNVEEIQPEQAETNKQRTDKN 50
p55 1  .MADQEKSQAQDAQNAAPQETAFVGMNGEETGLGFATRDQGAKEEDQGLL 49
Gcn5p 51  KGNFKETERIGGSSEVVTDEKGIYKFEFDGVEYTFKERFSVVEENEGKI 100
p55 50  DFDILTNDGTHRNMKLLIDLKNIFSRQLPKMPKEYIVKLVLDRHHESMVI 99
Gcn5p 101  EFRVVMNDNTRENNMVLTLGNIFQKQLPKMPKEYIARLVYDRSHLSMAV 150
p55 100  LKNKQKVIGGICFRQYKQRFQFAEVAFLAVTANEQVRGYGTRLMNKPKDHM 149
Gcn5p 151  IIRKPLTVGGITTYRPFDRKEFAEIVFCAISSTEQVRGYGAHLAMHLKIV 200
p55 150  QKQNIIEYLLTYADNFAIGYFKKQGFTKEHRMPQEKWKGYIKDYDGGTLME 199
Gcn5p 201  RNTSNIKYFLTYADNYAIGYFKKQGFTKEITLDKSIWGYIKDYEGGTLME 250
p55 199  CYIHPYVDYGNISQIIKQKELLIERIKKLSLNEKVFSGKEYAALIQNSM 248
Gcn5p 251  QCSLPRIRYLDAGKILLQEAALRRKIRTISKSHIVRPGLEQPKDLNMI 300
p55 249  DNEDPENPKVNPSPDIPGVAFSGWEWKDYHELKKSERSFNLCANVIGNM 298
Gcn5p 301  K.....FIDPMTIPGLKEAGWTPEMDALAQRPRKOPHDAIQNILTE 342
p55 299  KRHKQSWPFLDPVNDKDDVPDYDVITDPIIDIKAEKKLONNOYVDKDQFI 348
Gcn5p 343  LQNHAAWPFLLQPVNKEEVDDYDFLKEPMDLSTMEIKLESNKYQKMDP 392
p55 349  KDVKRIFTNAKIYNQPDITYYKAAKELEDFVEPYLTKLKDTPKESHTPSNN 398
Gcn5p 393  IYDARLVFNNCRMYNSENTSYKYANRLSKFFNNKVKELFYSKLLID... 439
p55 399  NSAHGSKKPLPVSQKKVQKRNE
  
```

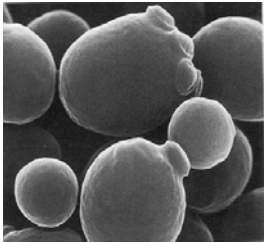
**Activity Gel**



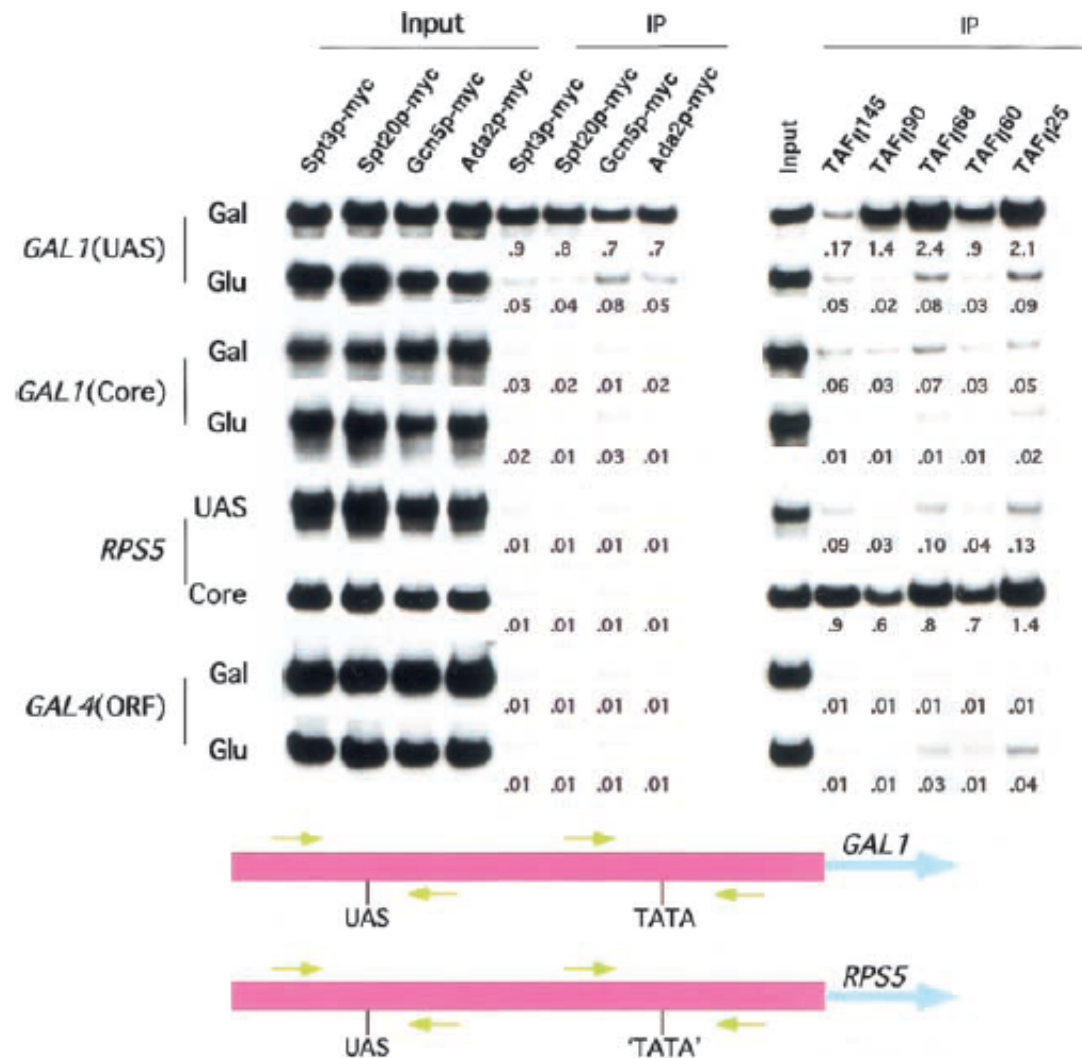


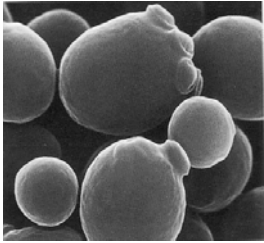
# Gcn5 Functions in Ada/SAGA Complexes



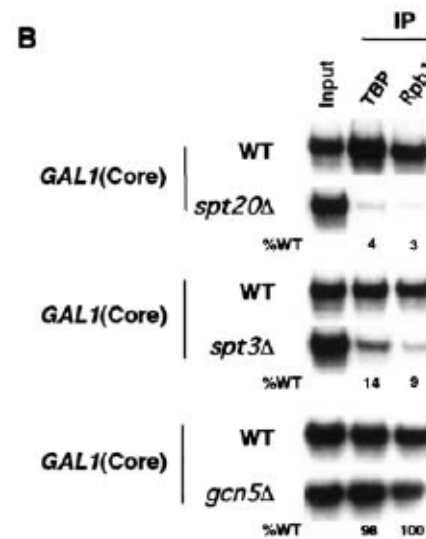
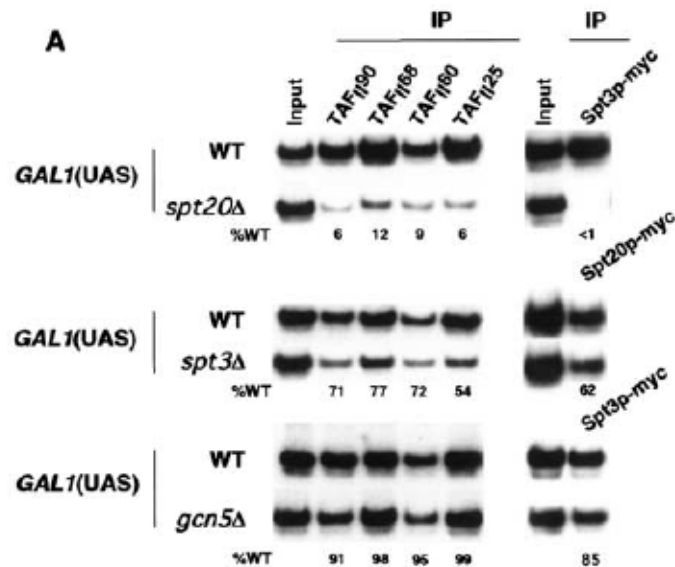
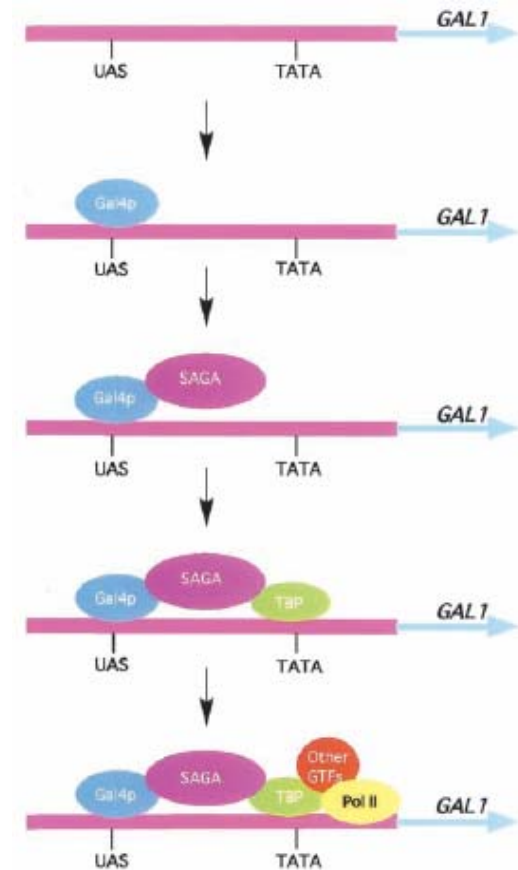
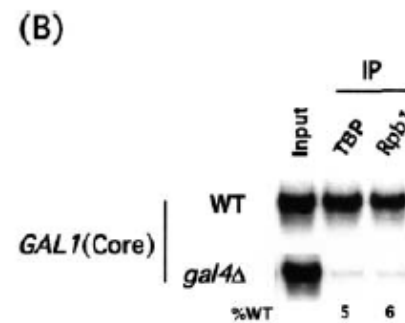
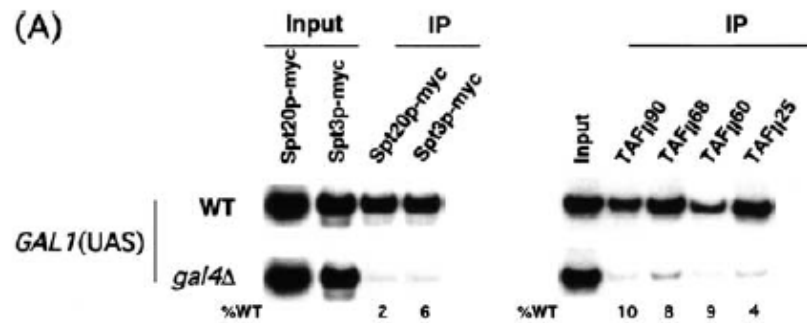


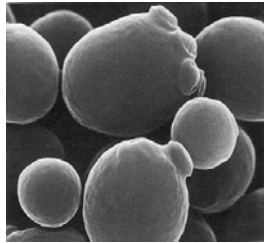
# SAGA is Recruited to Gal4p Activated Promoter Regions in Galactose Induction



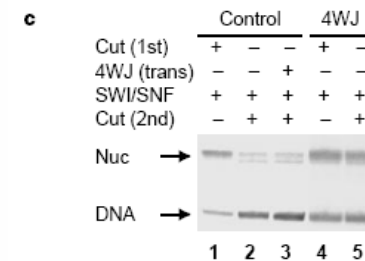
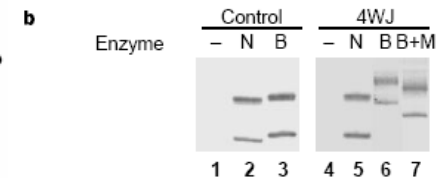
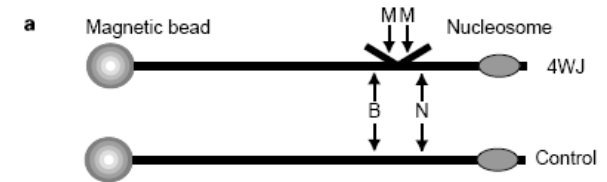
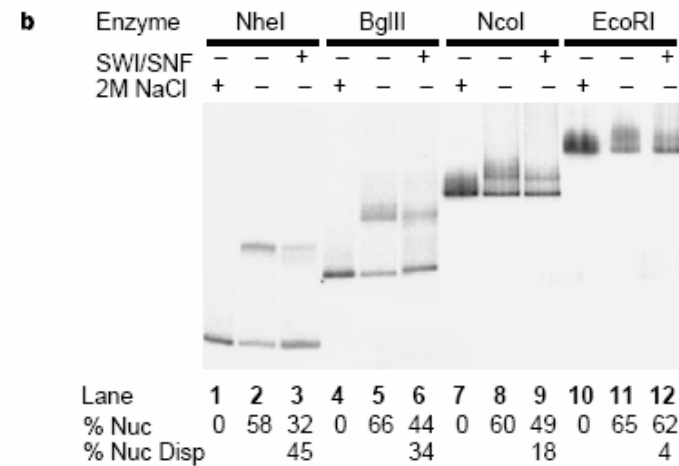
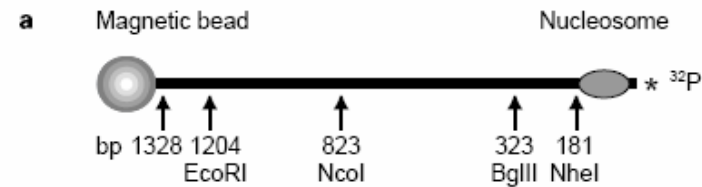
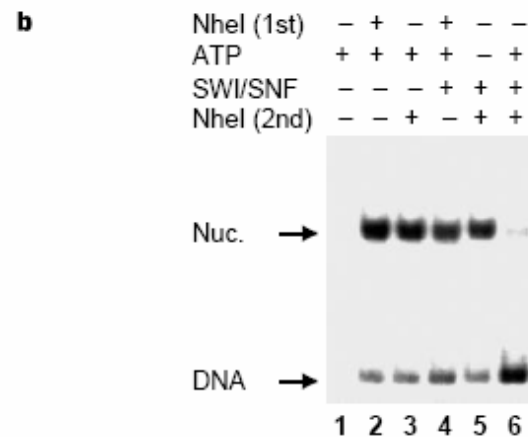
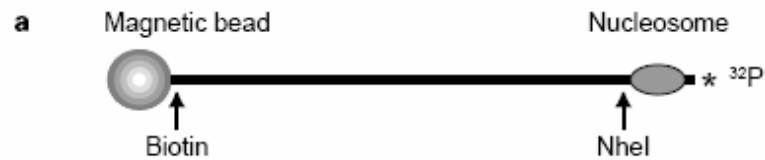


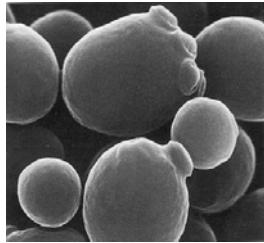
# SAGA is Recruited by Gal4p and is Required for Transcriptional Activation





# Swi/SNF is Required to Mobilize Nucleosome





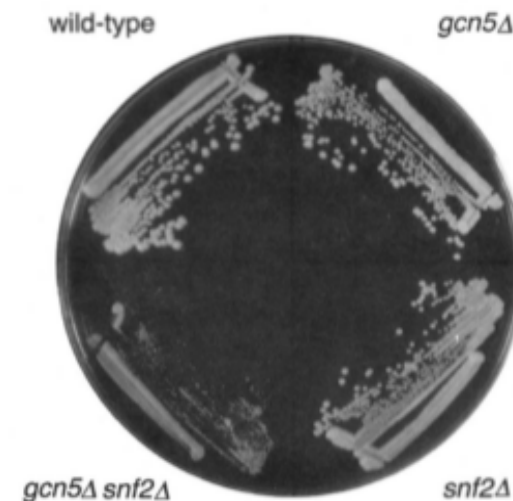
# Genetic Interactions between SAGA and Swi/Snf Complexes

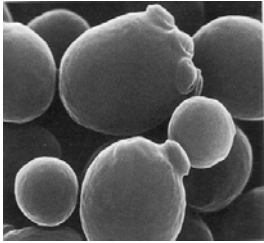
*spt20* and *spt7*, but not *spt3*, or *spt8* mutations are synthetically lethal with *snf/swi* and *srb/mediator* mutations

Double mutant <sup>a</sup>	Phenotype <sup>b</sup>
A. <i>spt20</i> Δ <i>snf2</i> Δ	Dead
<i>spt20</i> Δ <i>snf5</i> Δ	Dead
<i>spt20</i> Δ <i>swi1</i> Δ	Dead
<i>spt20</i> Δ <i>sin4</i> Δ	Dead
<i>spt20</i> Δ <i>gal11</i> Δ	Dead
<i>spt20</i> Δ <i>rgr1</i> Δ2	Dead
<i>spt20</i> Δ <i>srb2</i> Δ	Dead
<i>spt20</i> Δ <i>srb5</i> Δ	Dead
B. <i>spt7</i> Δ <i>snf2</i> Δ	Dead
<i>spt7</i> Δ <i>sin4</i> Δ	Dead
<i>spt7</i> Δ <i>gal11</i> Δ	Dead
C. <i>spt3</i> Δ <i>snf2</i> Δ	Alive
<i>spt3</i> Δ <i>sin4</i> Δ	Alive
<i>spt3</i> Δ <i>gal11</i> Δ	Alive
<i>spt3</i> Δ <i>rgr1</i> Δ2	Alive
<i>spt3</i> Δ <i>srb2</i> Δ	Alive
D. <i>spt8</i> Δ <i>snf2</i> Δ	Alive
<i>spt8</i> Δ <i>sin4</i> Δ	Alive
<i>spt8</i> Δ <i>gal11</i> Δ	Alive

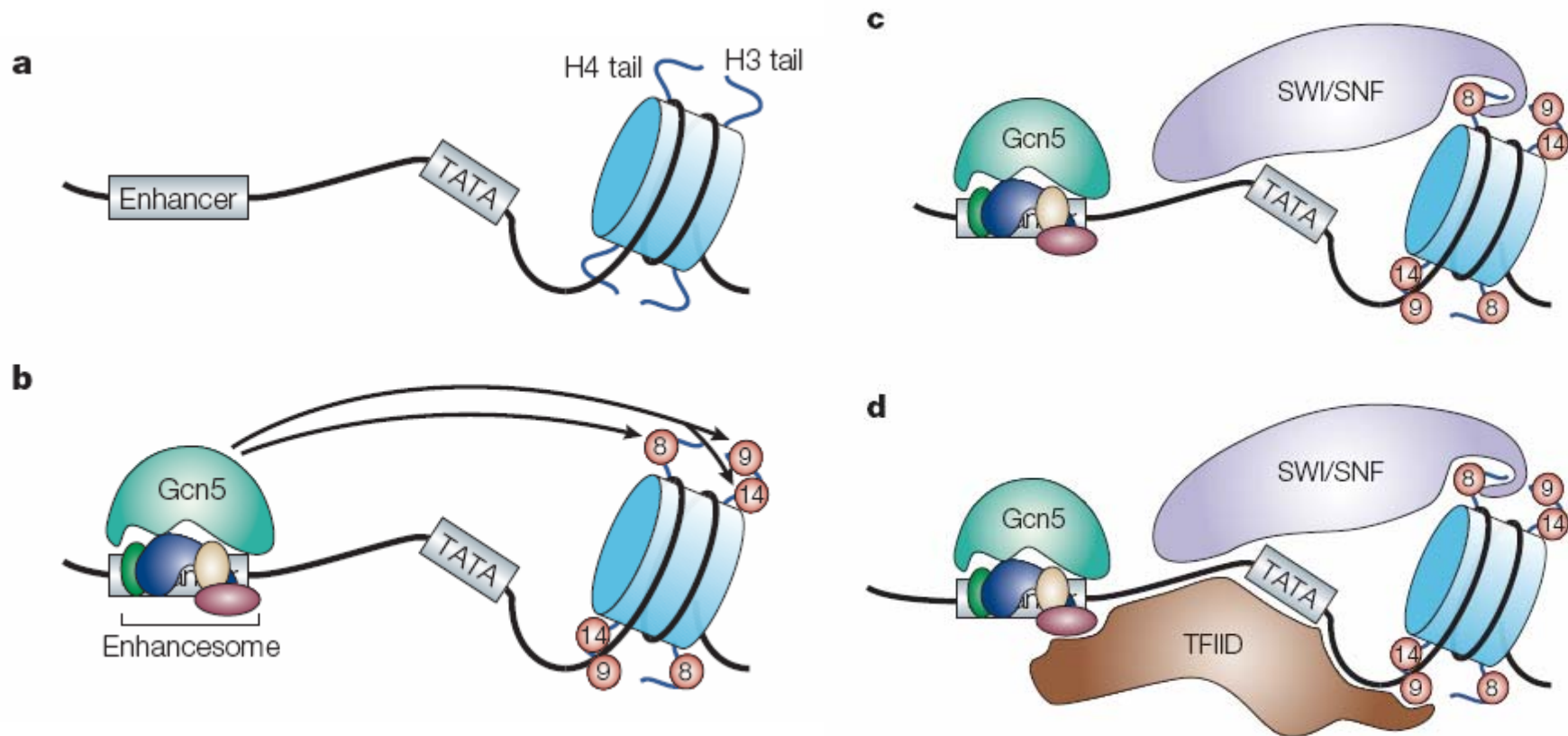
Genetic interactions between *gcn5*Δ, *srb/mediator*, *snf/swi* and *spt* mutants

Double mutant <sup>a</sup>	Phenotype <sup>b</sup>
<i>gcn5</i> Δ <i>sin4</i> Δ	Alive
<i>gcn5</i> Δ <i>srb2</i> Δ	Alive
<i>gcn5</i> Δ <i>snf5</i> Δ	Alive, sick
<i>gcn5</i> Δ <i>swi1</i> Δ	Alive, sick
<i>gcn5</i> Δ <i>snf2</i> Δ	Alive, sick
<i>gcn5</i> Δ <i>spt3</i> Δ	Alive
<i>gcn5</i> Δ <i>spt7</i> Δ	Alive
<i>gcn5</i> Δ <i>spt20</i> Δ	Alive

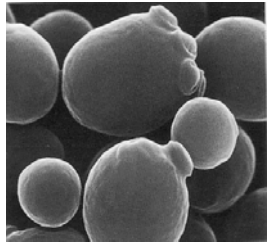




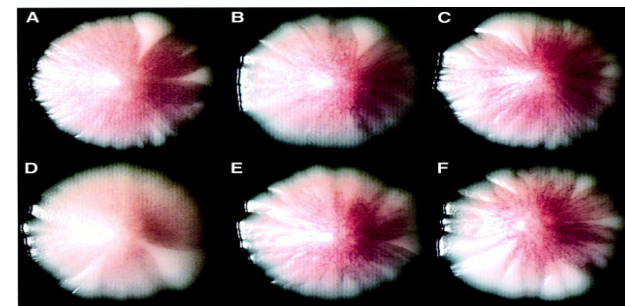
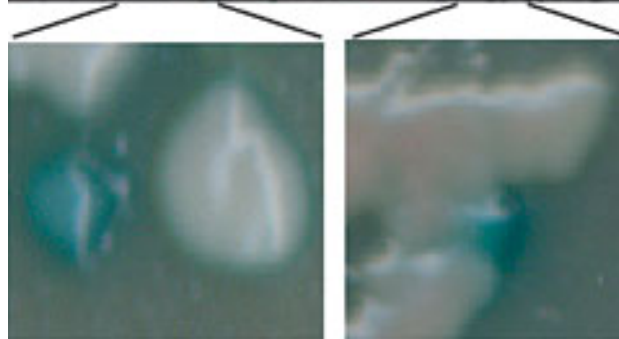
# SAGA Functions Together with Swi/SNF Complex to Mobilize Acetylated Nucleosome



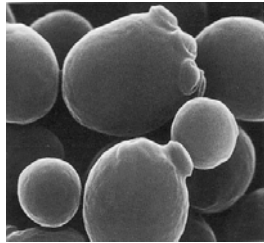




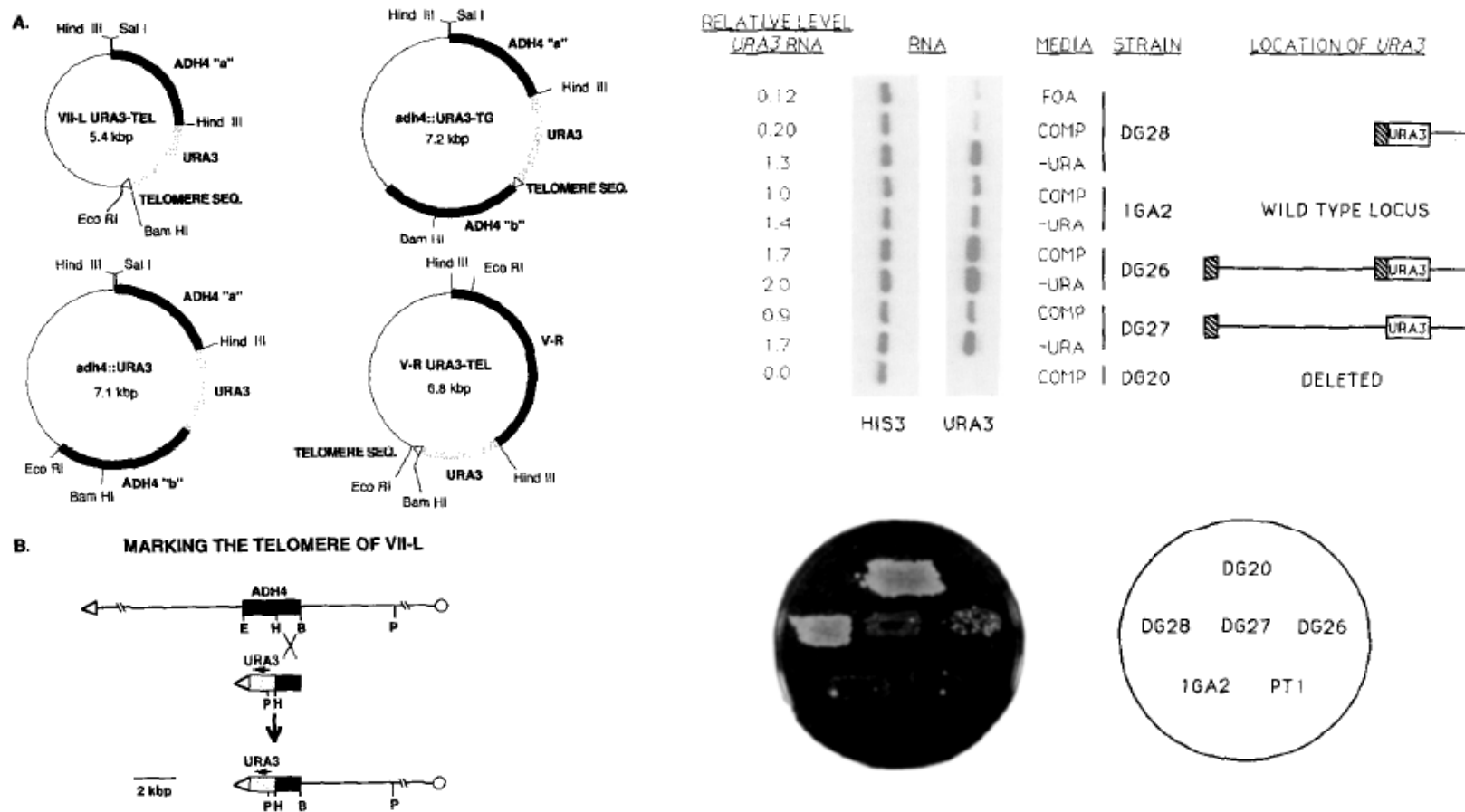
# Phenotypic Variegation at the Level of Transcriptional Efficacy

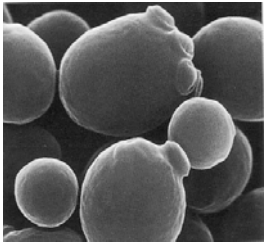




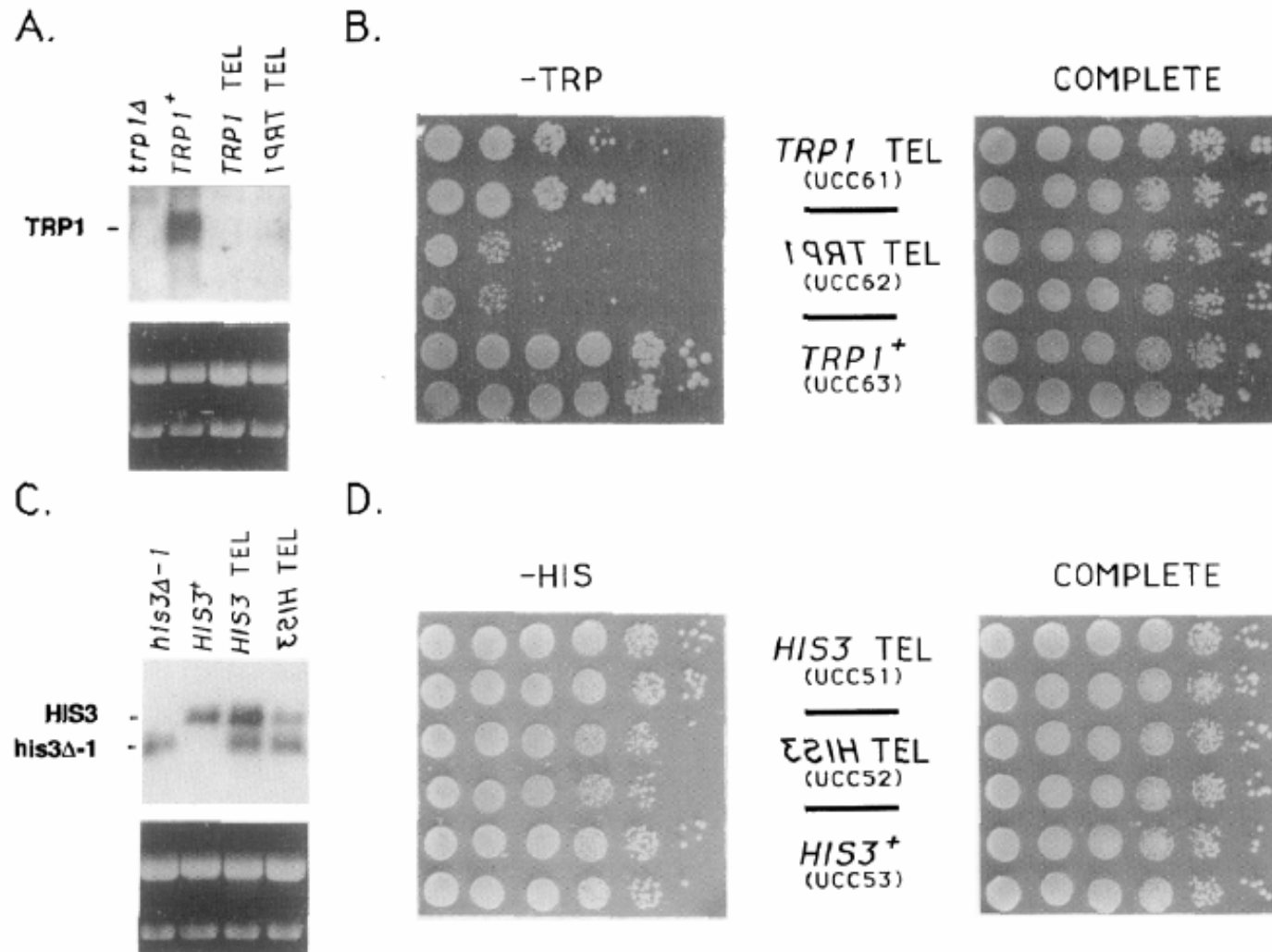


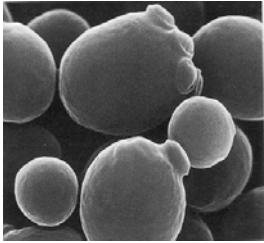
# Telomeric Effect of Silencing



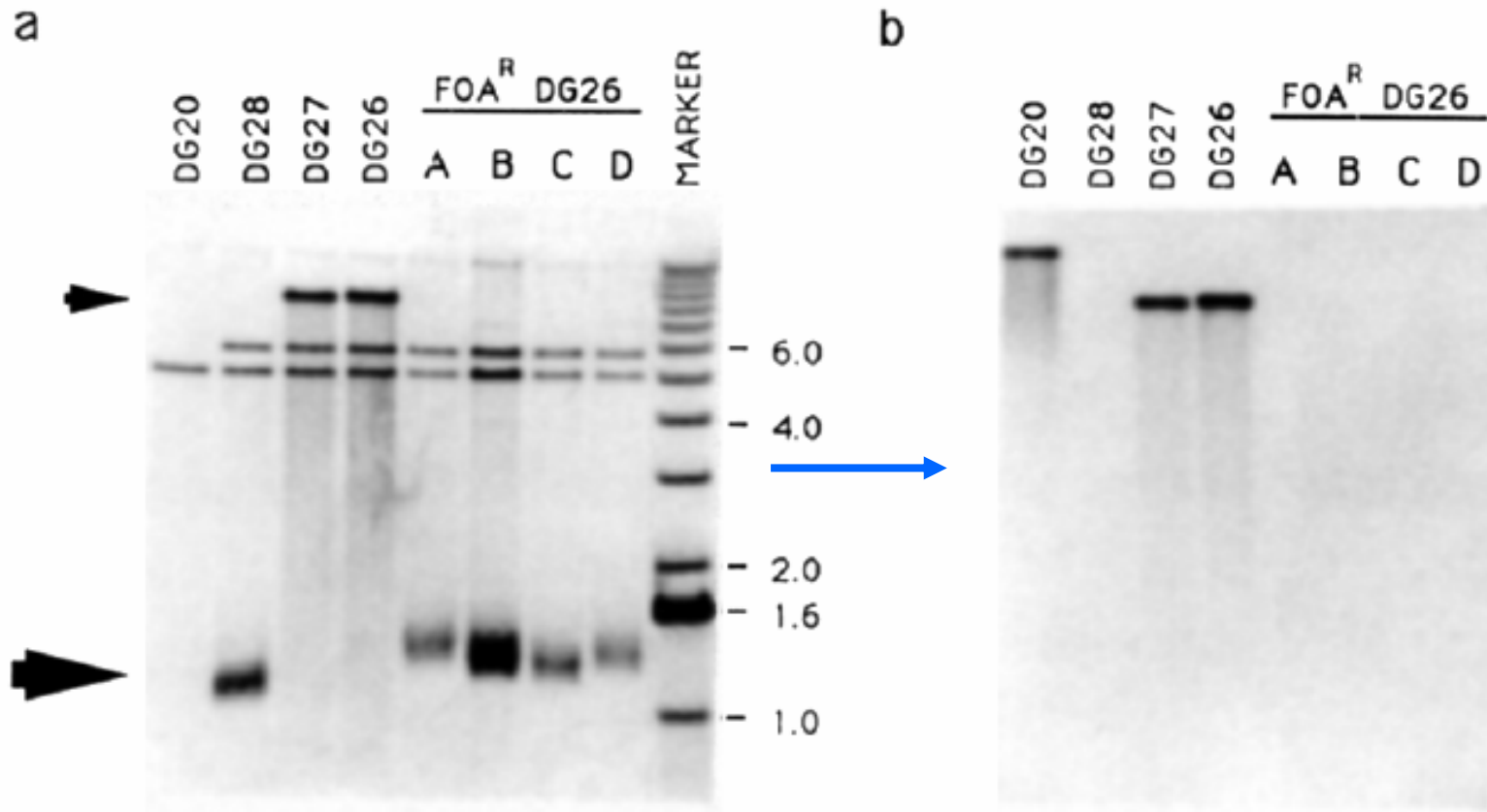


# Telomeric Effect of Silencing



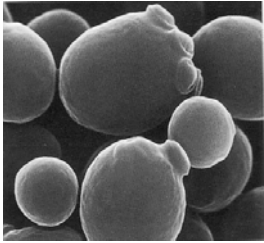


# Telomeric Effect of Silencing

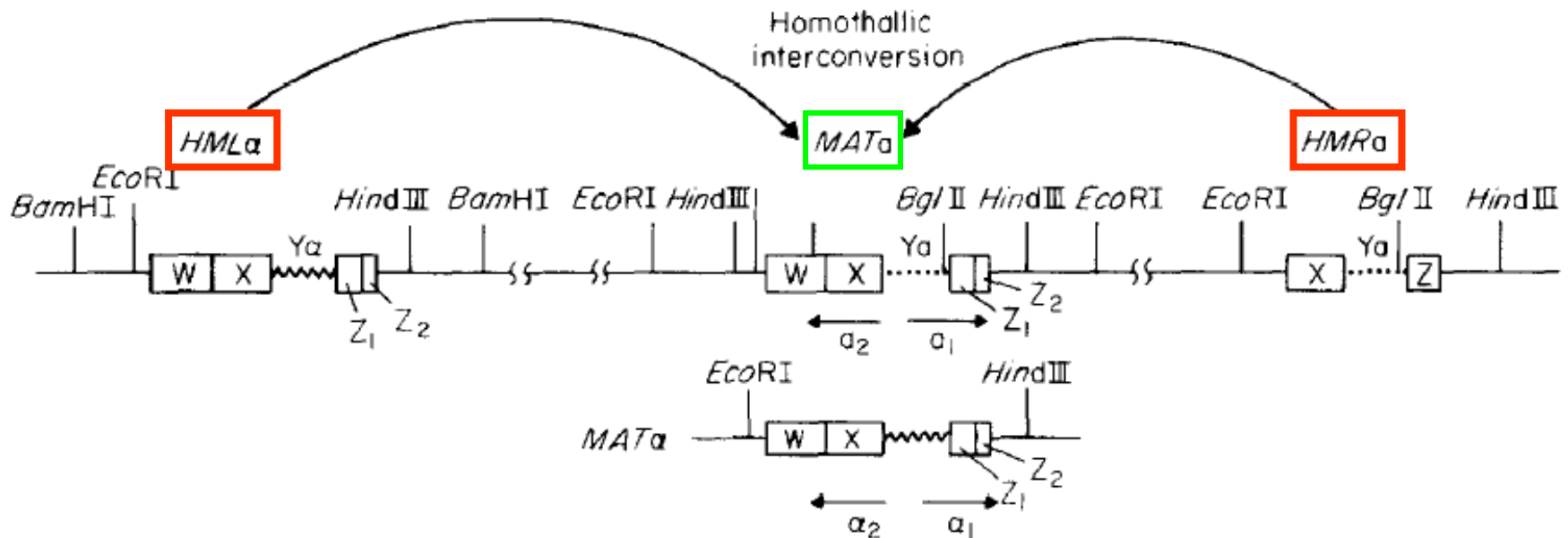


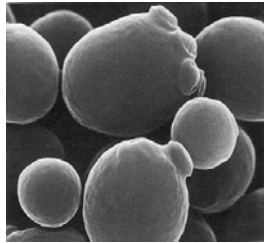
Southern  
PstI cut genomic, URA3 probe

Northern

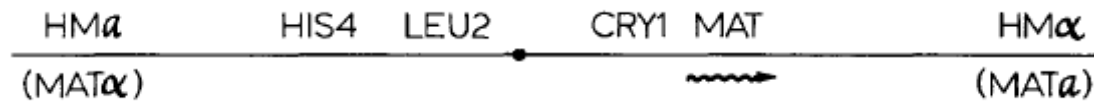


# Mating Type Gene Loci





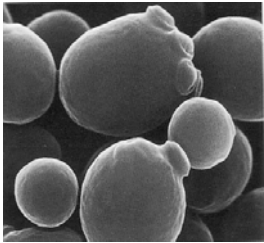
# Mating Type Suppression by SIR



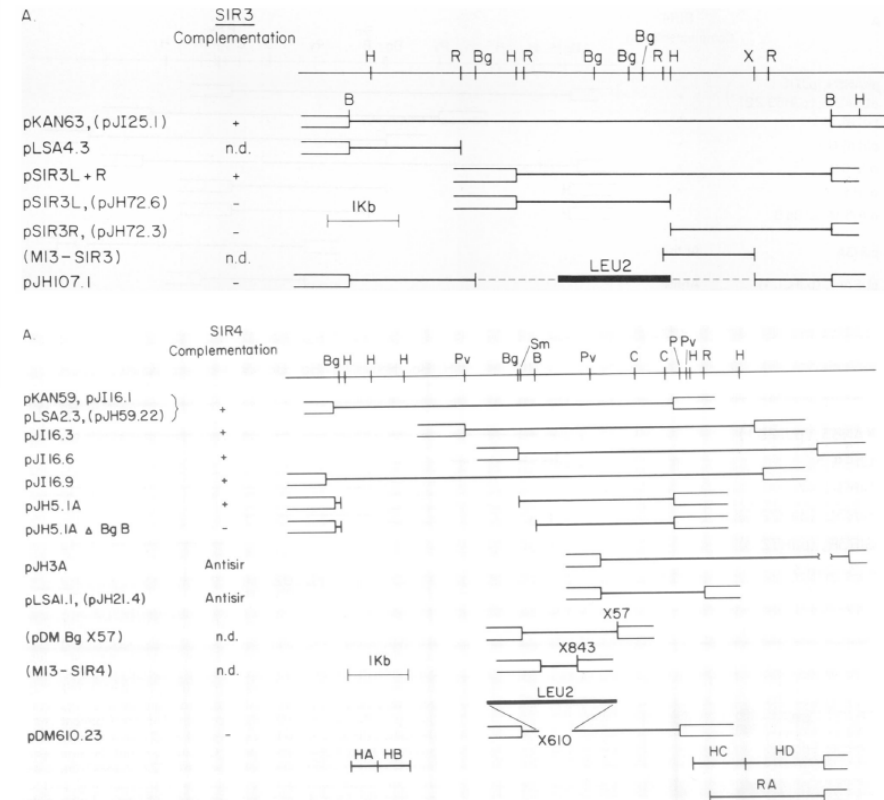
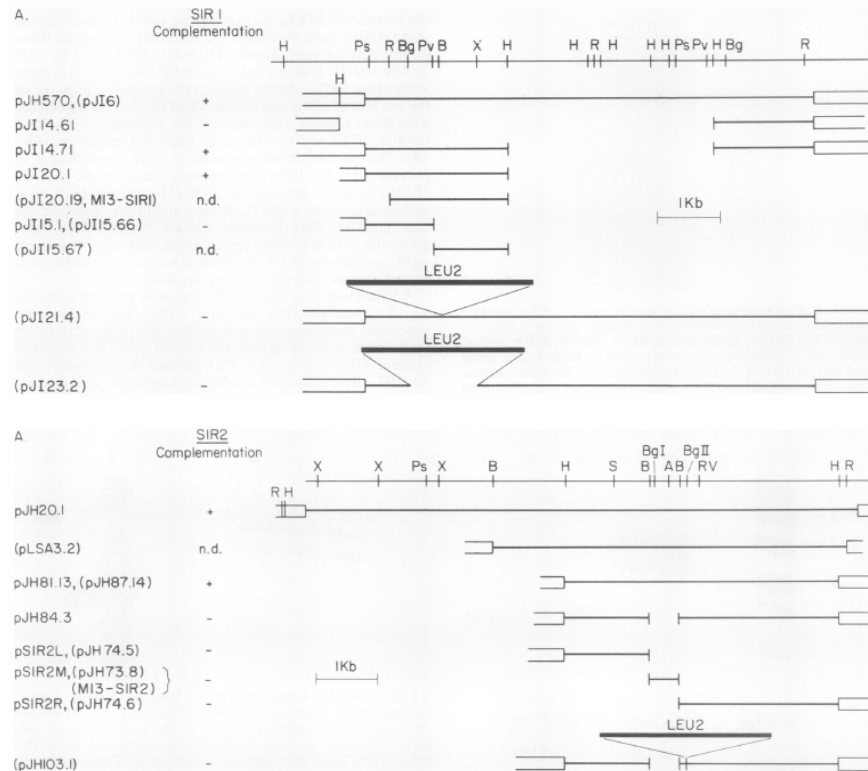
*Suppression of the mating defect of mata1-5, mata2-4, and mata2-1 by sir1-1*

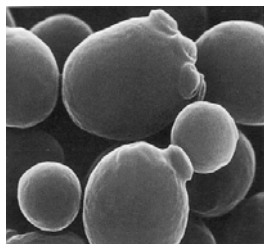
Diploid	Genotype				PD	Tetrad Types* NPD	T
XR202	<i>CRY1</i>	<i>mata1-2</i>	<i>SIR1</i>		13	14	51
	<i>cry1-3</i>	<i>MATa</i>	<i>sir1-1</i>				
XS8E	<i>CRY1</i>	<i>mata1-2</i>	<i>SIR1</i>	<i>rme</i>	1	2	3
	<i>cry1-3</i>	<i>mata1-5</i>	<i>sir1-1</i>	<i>csp1</i>			
XR128E	<i>CRY1</i>	<i>mata2-4</i>	<i>SIR1</i>		13	21	52
	<i>cry1-3</i>	<i>MATa</i>	<i>sir1-1</i>				
XS5F	<i>CRY1</i>	<i>mata2-4</i>	<i>SIR1</i>	<i>rme</i>	2	2	4
	<i>cry1-3</i>	<i>mata1-5</i>	<i>sir1-1</i>	<i>csp1</i>			
XJ111	<i>CRY1</i>	<i>mata2-4</i>	<i>SIR1</i>	<i>rme</i>	1	5	8
	<i>cry1-3</i>	<i>mata1-5</i>	<i>sir1-1</i>	<i>csp1</i>			
XJ104	<i>CRY1</i>	<i>mata2-1</i>	<i>SIR1</i>	<i>rme</i>	1	3	5
	<i>cry1-3</i>	<i>mata1-5</i>	<i>sir1-1</i>	<i>csp1</i>			
XJ110	<i>CRY1</i>	<i>mata2-1</i>	<i>SIR1</i>	<i>rme</i>	4	1	10
	<i>cry1-3</i>	<i>mata1-5</i>	<i>sir1-1</i>	<i>csp1</i>			

\* For XR202 and XR128E, PD = 2 nm : 2 a; NPD = 2  $\alpha$  : 2 a; T = 1  $\alpha$  : 1 nm : 2 a. For other crosses, PD = 2 CryR  $\alpha$  : 2 CryS nm; NPD = 2 CryR nm : 2 CryS  $\alpha$ ; T = 1 CryR  $\alpha$  : 1 CryS  $\alpha$  : 1 CryR nm : 1 CryS nm.

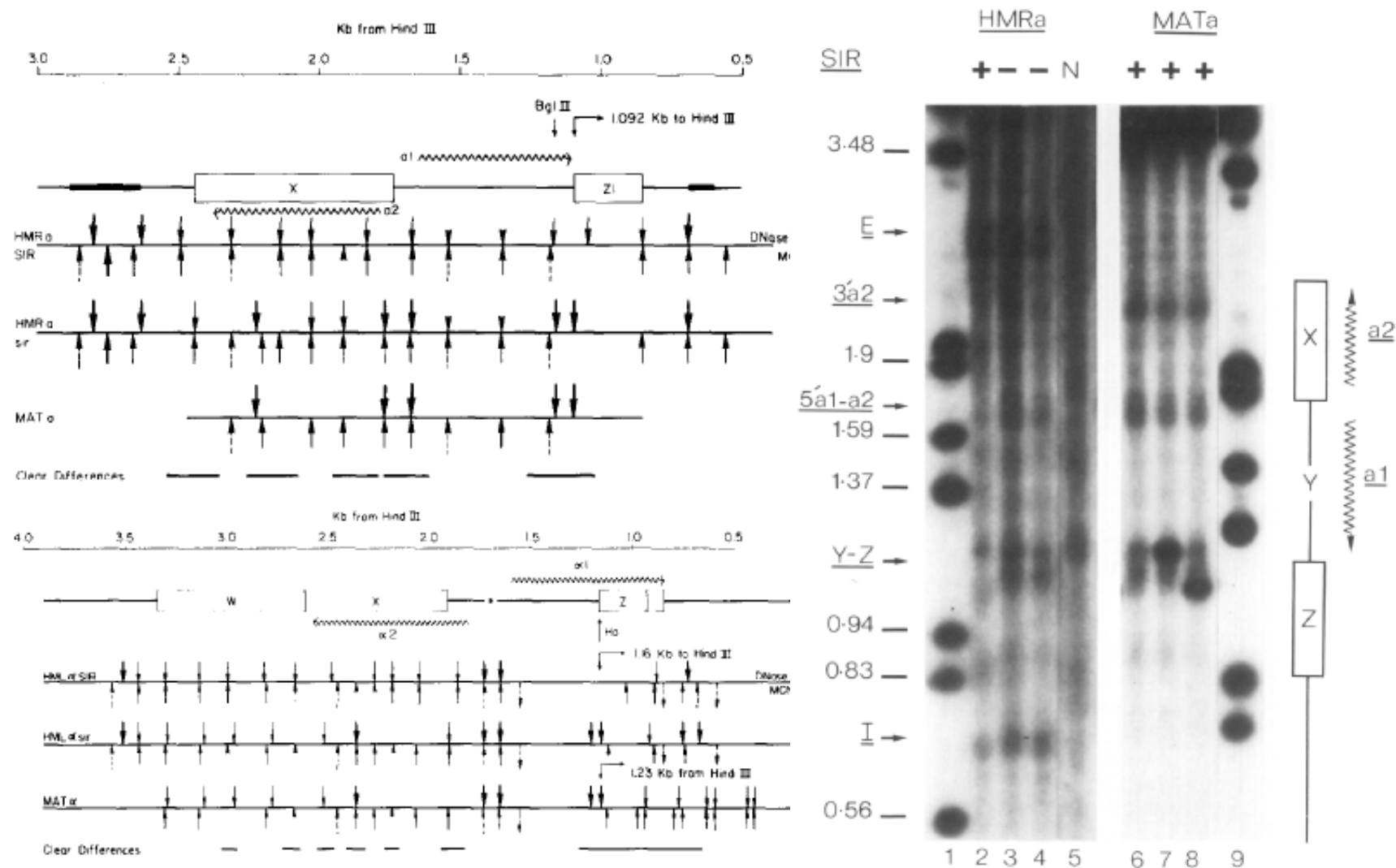


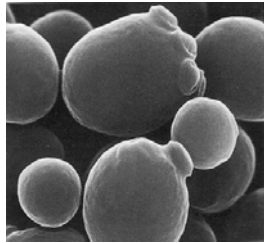
# Cloning of SIR1-4



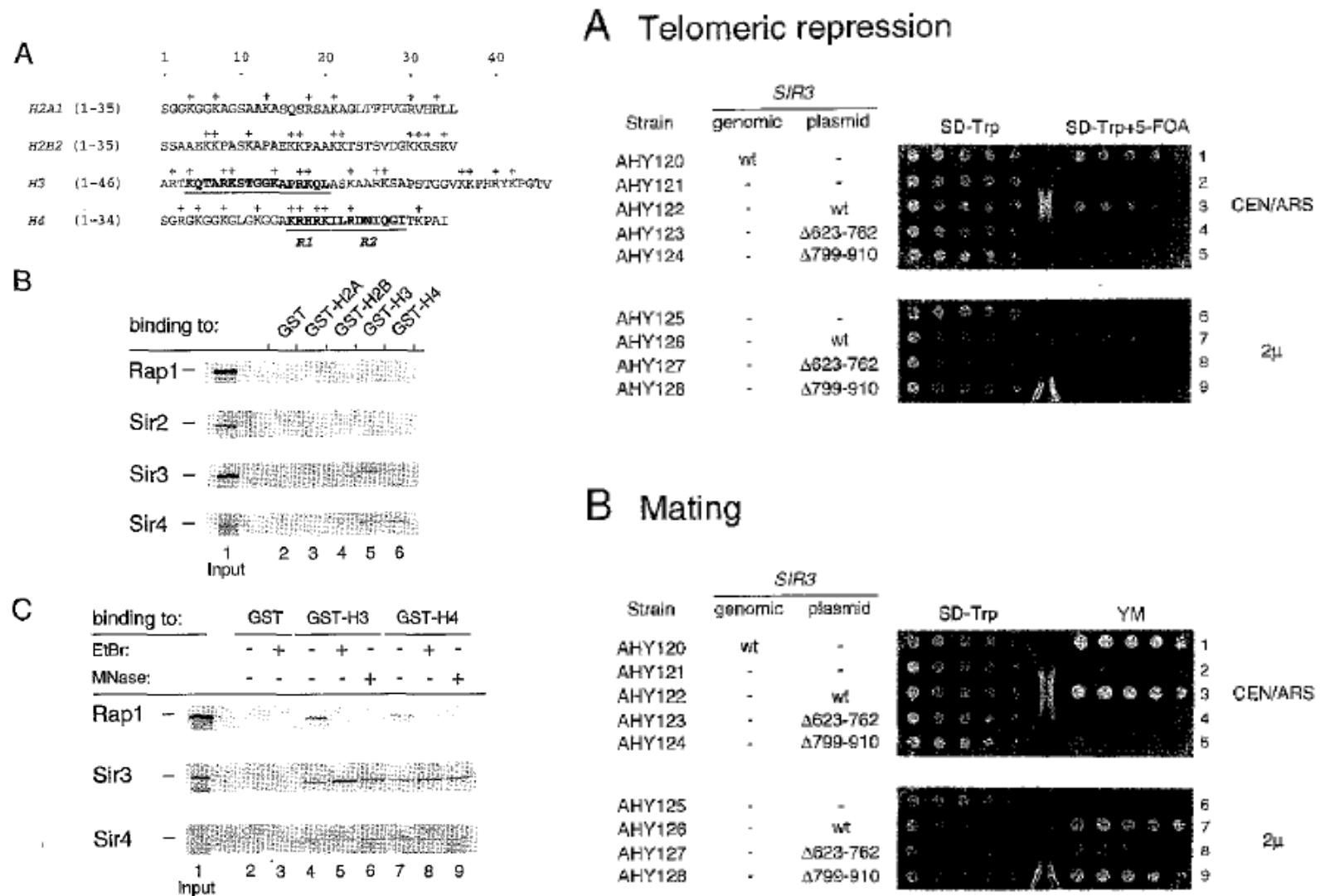


# SIR Regulates Heterochromatin Structure at HML and MAT Loci

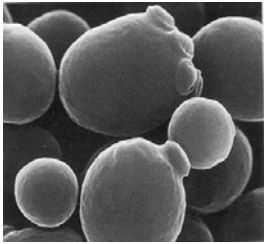




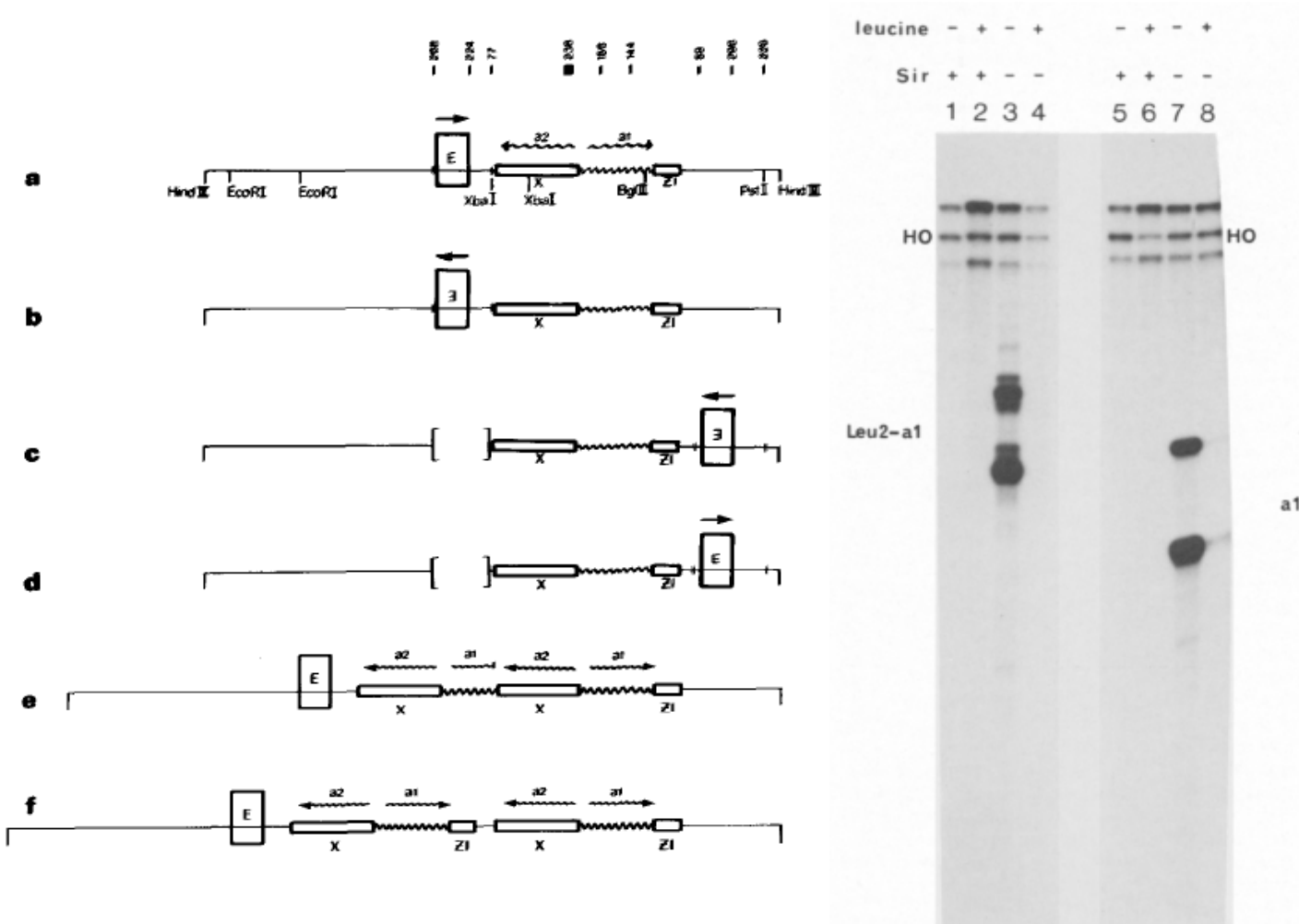
# SIR3/4 Binds to H3/4 N-termini

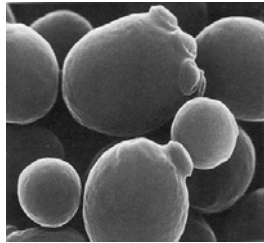




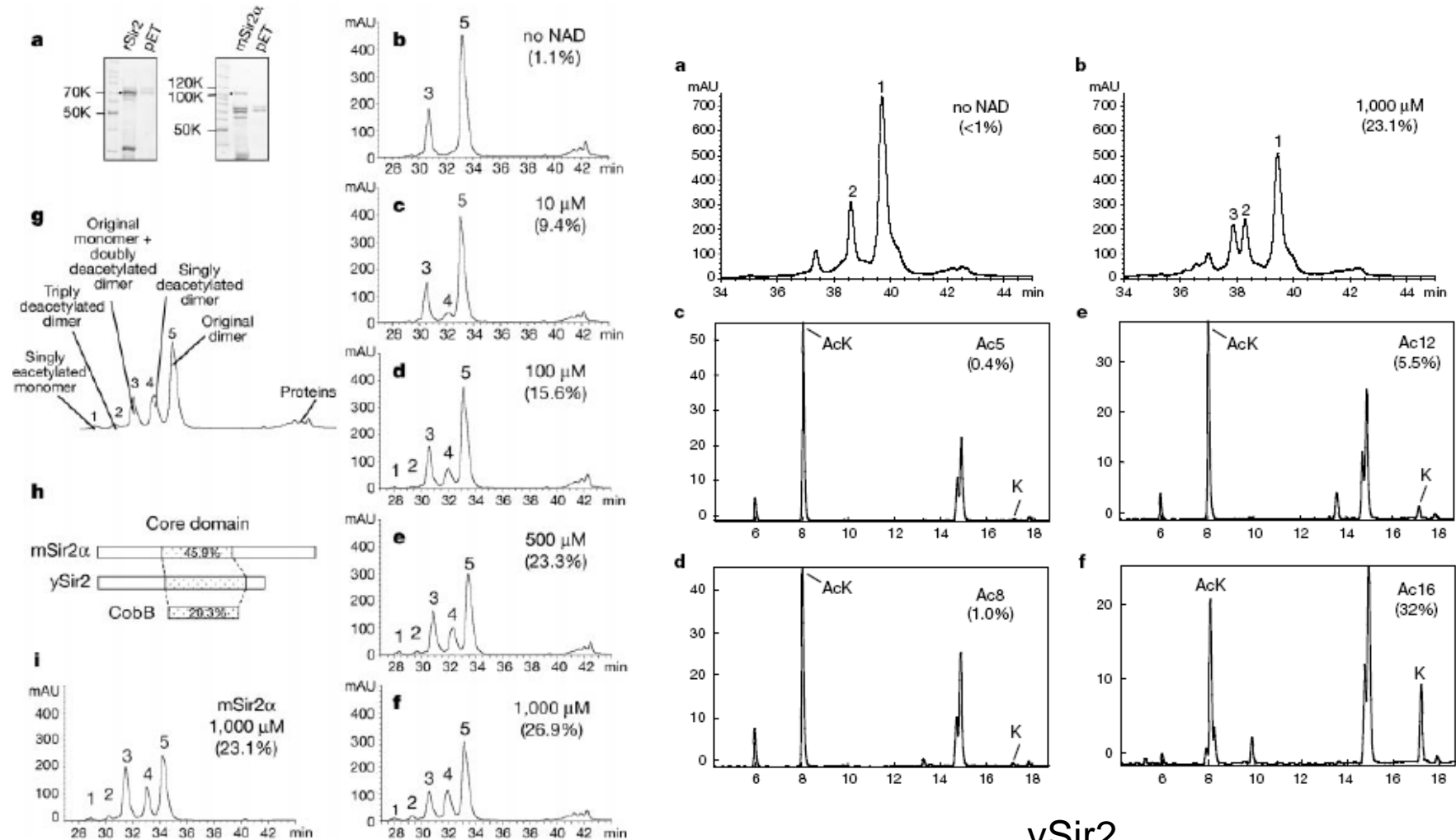


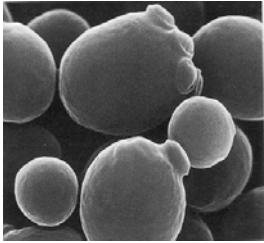
# Chromatin Suppressor Binding Site for SIR Complexes



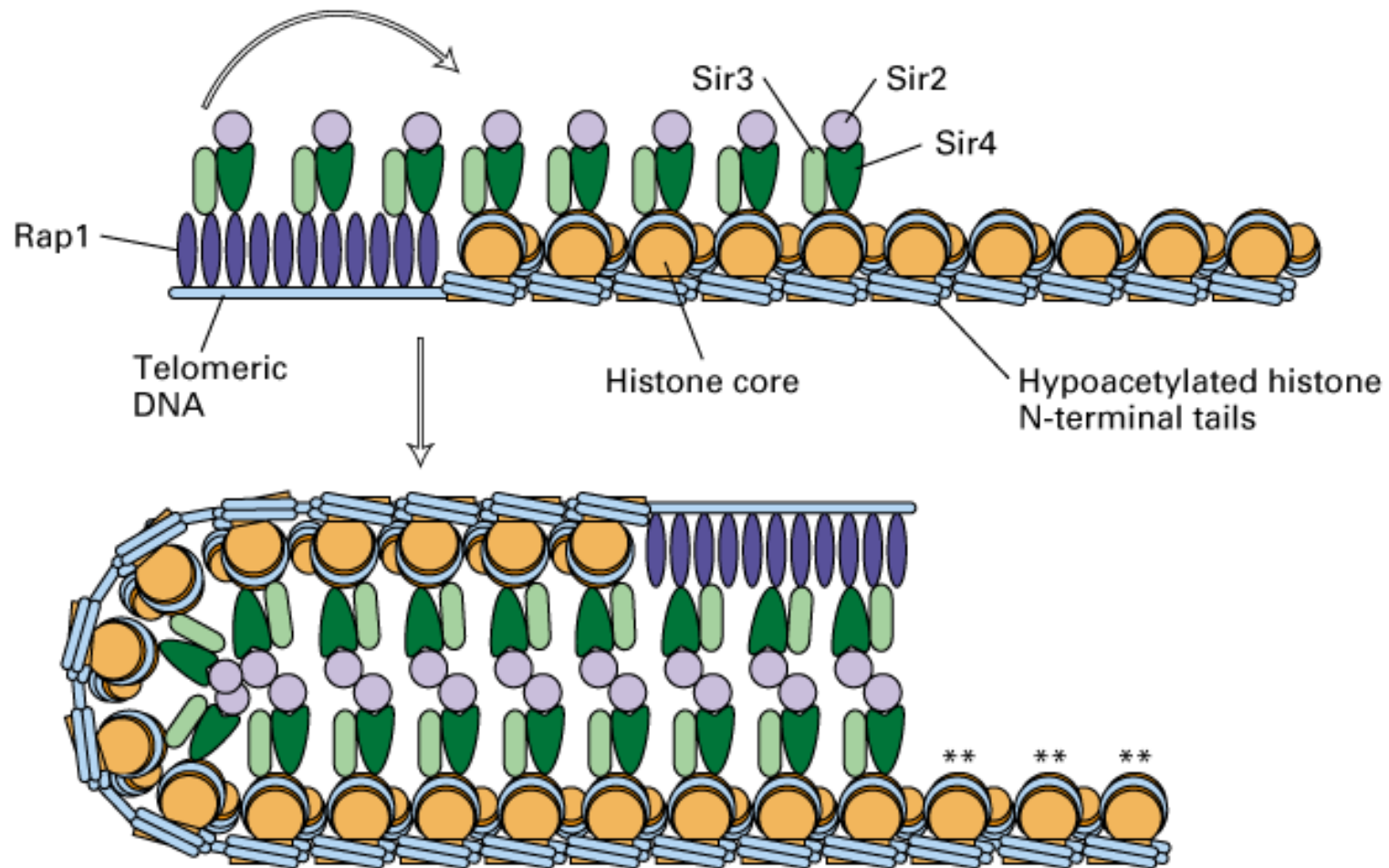


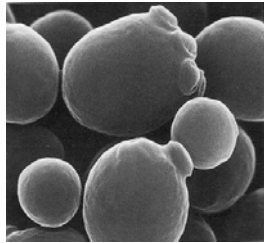
# SIR2 Proteins are HDACs





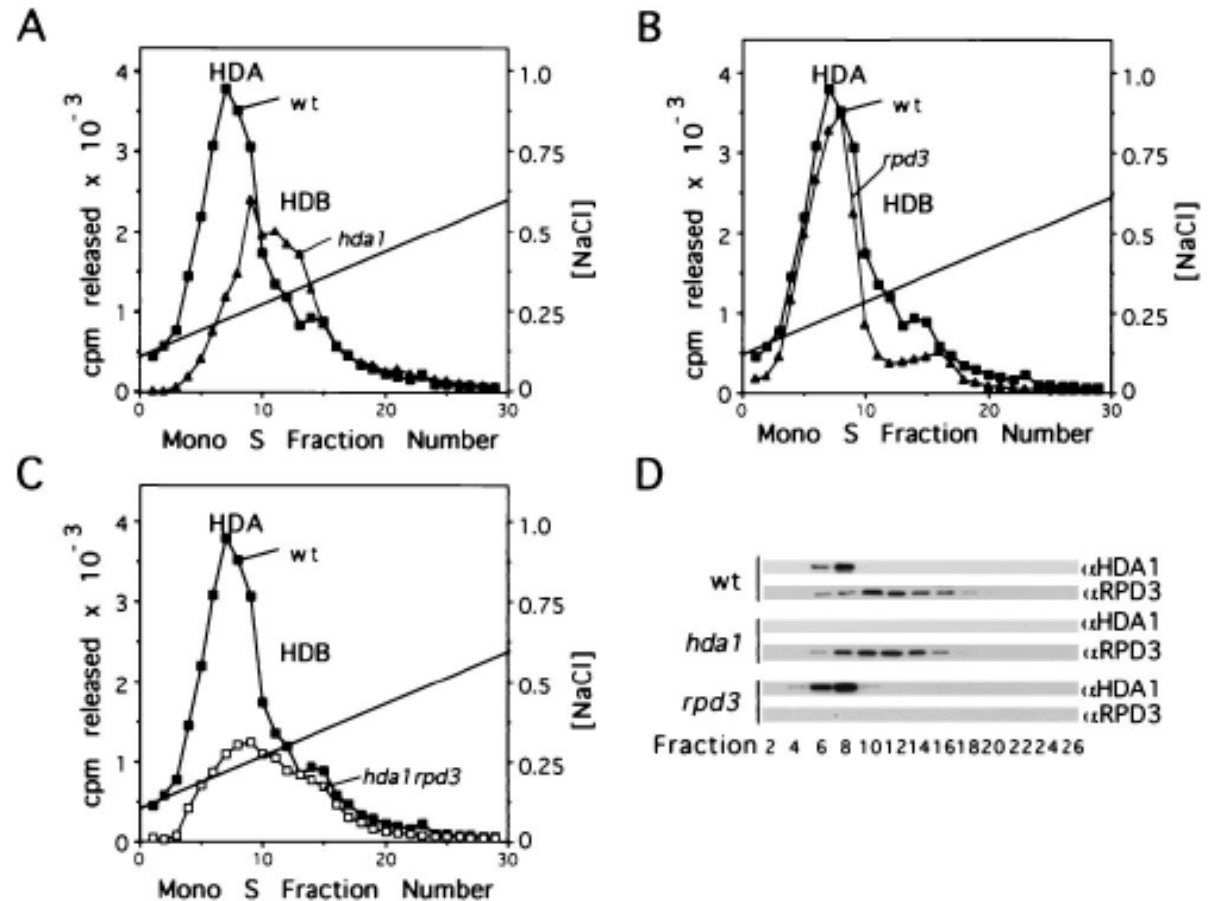
# SIR2/3/4 Complex Formation During Heterochromatin Assembly

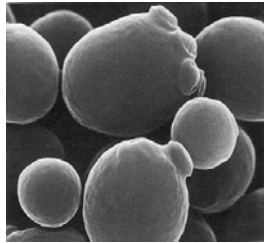




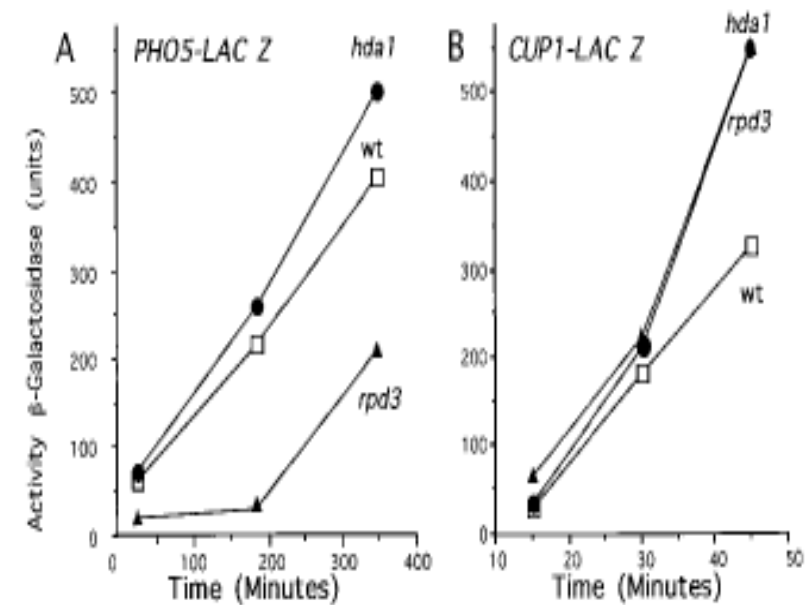
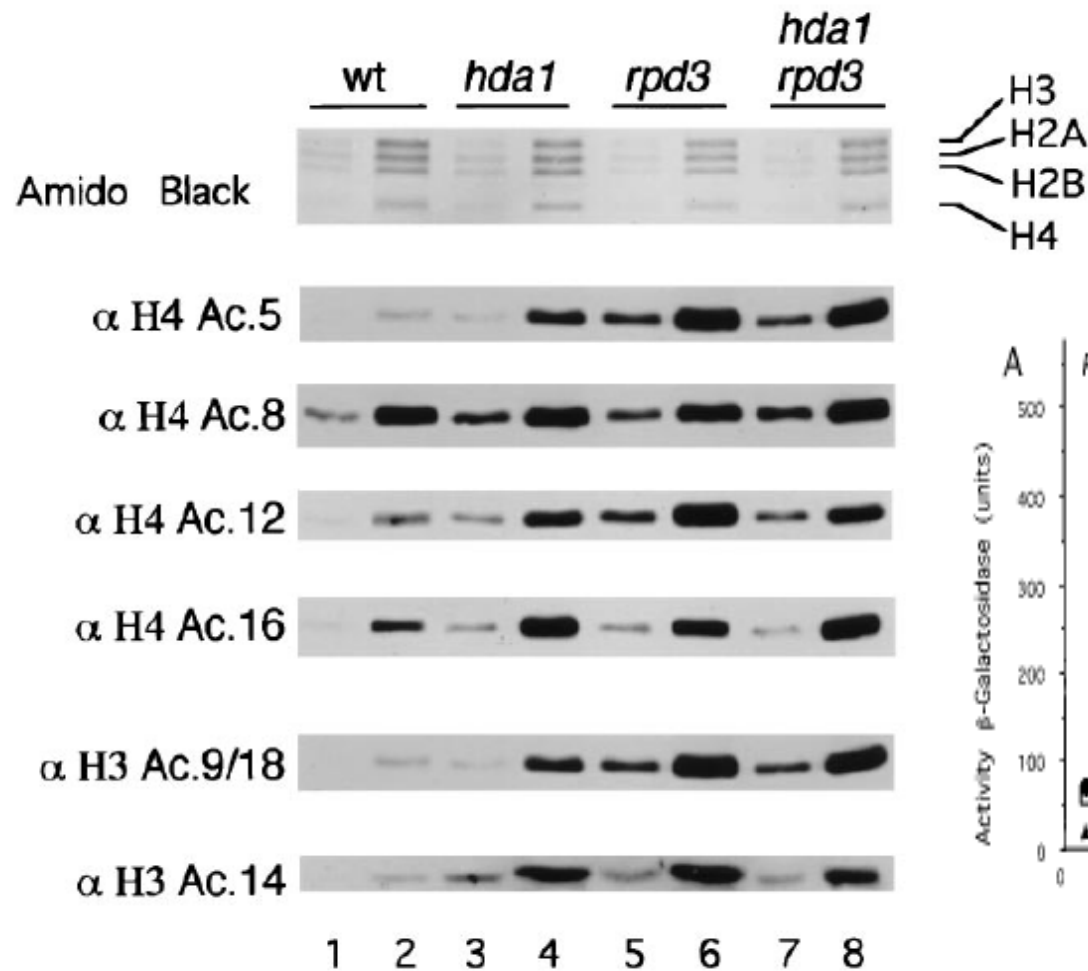
# Biochemistry Assays Identified Rpd3/Hos2/Hda1 in different HDAC Complexes

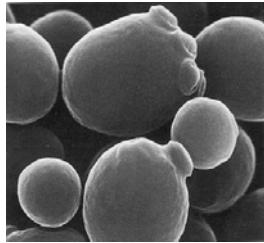
HDA1 64 SPLKGTGLCYDVHRRHAKIFZSYFPCDPPHPTTYREYKLAENFLIDDP  
 RPD3 9 DPITVKEPSDEKRYVAVDADGGMVAGAGHHEHGLIRMANSEINMTGLYKK-  
 HOS1 2 SKLVISTE-----IPQSGVADLLCHNHG-----LQSLYVGLCNANGLQH-  
 HOS2 10 --FEPNAYSPDGVHRRHAKIFZSYFPCDPPHPTTYREYKLAENFLIDDP  
 HOS3 1 -----NSKKHSDPLERTKQFQAFVQHHPHVISAA  
  
 HDA1 107 TLEGGVDDLDLHLKIPVVAATHRRHAKIFZSYFPCDPPHPTTYREYKLAENFLIDDP  
 RPD3 61 -----NEEYKAKHAKKQKTC-----CHHAKSVLDEI-----  
 HOS1 43 -----FOEVLTFVAAKDDDLLETHHSEVDDYHENGKPKRMAQGVNH-  
 HOS2 66 -----NDLVETREAAHIDDLCHHAKSVLDEI-----  
 HOS3 31 NAAAQIFESAKAVVVLGSLVSLQVFPFRRVYHNSKRTIV-RRTERLLASGGI  
  
 HDA1 147 EKESEETED-----LKEETRRDGYVYVNHGTAARLFCGGAIEA  
 RPD3 95 EHFKEEETV-----KTFVADGCPDGLHCKGSIAGHSMES  
 HOS1 104 DGLHAKHPPENDEPFTYTLNSETQVLAACPCITGVYHLCGVSTRAFLNL  
 HOS2 102 HPLLEKGL-----ENHSEEDCCTHCKLHTTLTVAHLDA  
 HOS3 131 AKLEKGL-----EVPDTUNSGSEVLSGXTEKALOGGTGATETG  
  
 HDA1 185 CKAVVEEAVENSIA-----VMTFTG-MHAKFOAAHFFCFPRVAVAAKNILEY  
 RPD3 131 AARLHKEE-KCDVA-----LVYAGGLHNAKESASGFCY-RDGVLLHITELDFA  
 HOS1 191 LDRHSEFT-KKLGL-----LHMDGGHNAKESASGFCY-RDGVLLHITELDFA  
 HOS2 139 TRSLHKL-QSDTA-----LHMDGGHNAKESASGFCY-RDGVLLHITELDFA  
 HOS3 170 VDSIFVPSAEHLHRRATGIRFPFGCHTGTTPRGGGLHNAVAAG--YAKD  
  
 HDA1 233 PRSVREINLLNCHHGGGGL-----LSPFYDDGQ  
 RPD3 178 -----PLVLYEDDPLHHGGGGL-----LSPFYDDGQ  
 HOS1 238 ---LNHLYVDDPLHHGGGGL-----LSPFYDDGQ  
 HOS2 194 -----PLVLYEDDPLHHGGGGL-----LSPFYDDGQ  
 HOS3 221 TYNVTHVVLHGGGGLHGGGGLICWKEAGTRFEEFSDSSYDDFQDGLASFPE  
  
 HDA1 262 PLVLYEDDPLHHGGGGL-----LSPFYDDGQ  
 RPD3 203 LKTLSPH---KYL-GRFFPGT-GRADIDNGAKENTAV-RNGLN-DGDDA7  
 HOS1 264 LKTLSPH---KYL-GRFFPGT-GRADIDNGAKENTAV-RNGLN-DGDDA7  
 HOS2 211 LKTLSPH---KYL-GRFFPGT-GRADIDNGAKENTAV-RNGLN-DGDDA7  
 HOS3 274 LKTLSPH---KYL-GRFFPGT-GRADIDNGAKENTAV-RNGLN-DGDDA7  
  
 HDA1 315 ---PKAAEQVQVNGRGLPLVLIASVDAADP-----PITDCHYTPSECT  
 RPD3 247 ---LRSVLPVILKINMYTPSAAVVLQCGGDESSD-----DRLGPHLRKEGH  
 HOS1 306 ---LRSVLPVILKINMYTPSAAVVLQCGGDESSD-----DRLGPHLRKEGH  
 HOS2 256 ---LRSVLPVILKINMYTPSAAVVLQCGGDESSD-----DRLGPHLRKEGH  
 HOS3 327 LKTLSPH---KYL-GRFFPGT-GRADIDNGAKENTAV-RNGLN-DGDDA7  
  
 HDA1 356 QHN-DHRLGL-----ARHGCVLGGGTHLDAIARHLSVAK  
 RPD3 292 AN-CLVYVGL-----G-IPNMLVGGGTHLDAIARHLSVAK  
 HOS1 351 SHILHAKHGL-----PRAHIFLGGGTHLDAIARHLSVAK  
 HOS2 301 GE-CLVYVGL-----G-IPNMLVGGGTHLDAIARHLSVAK  
 HOS3 380 QRHSHVPTETTTTTRDALKLAQNRCHGVLSLNRGGVDRATC--GQVPAH



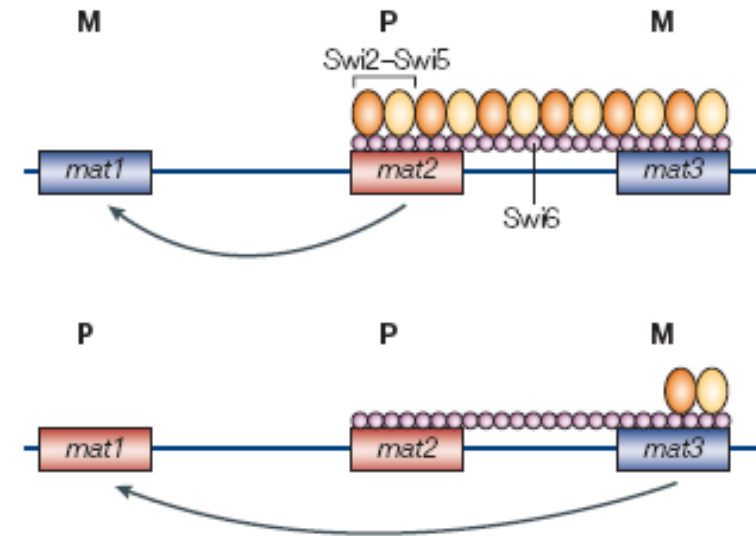
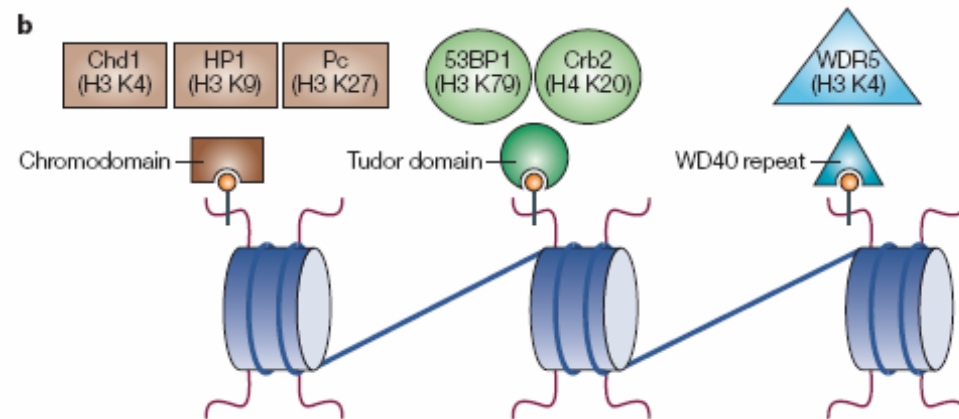
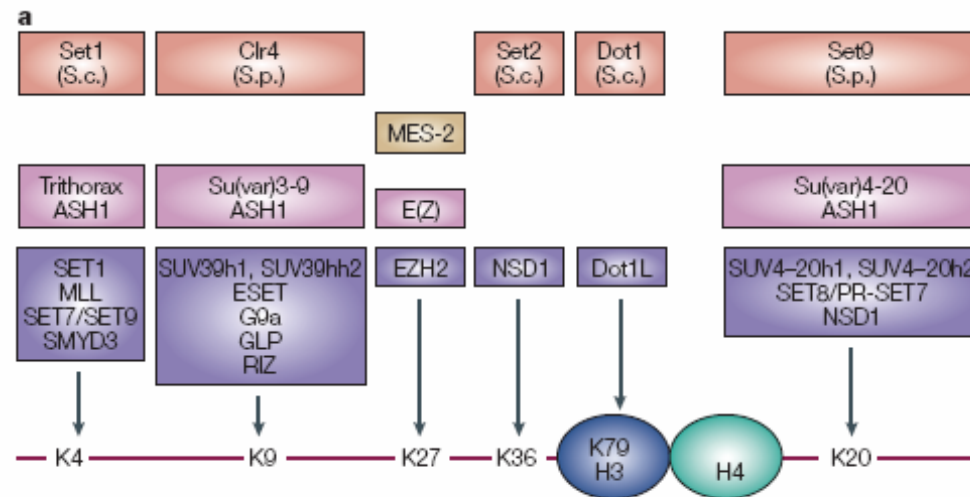


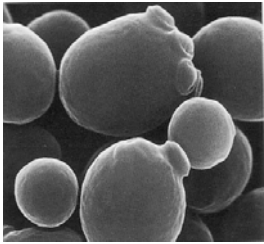
# Differential Activity of HDACs



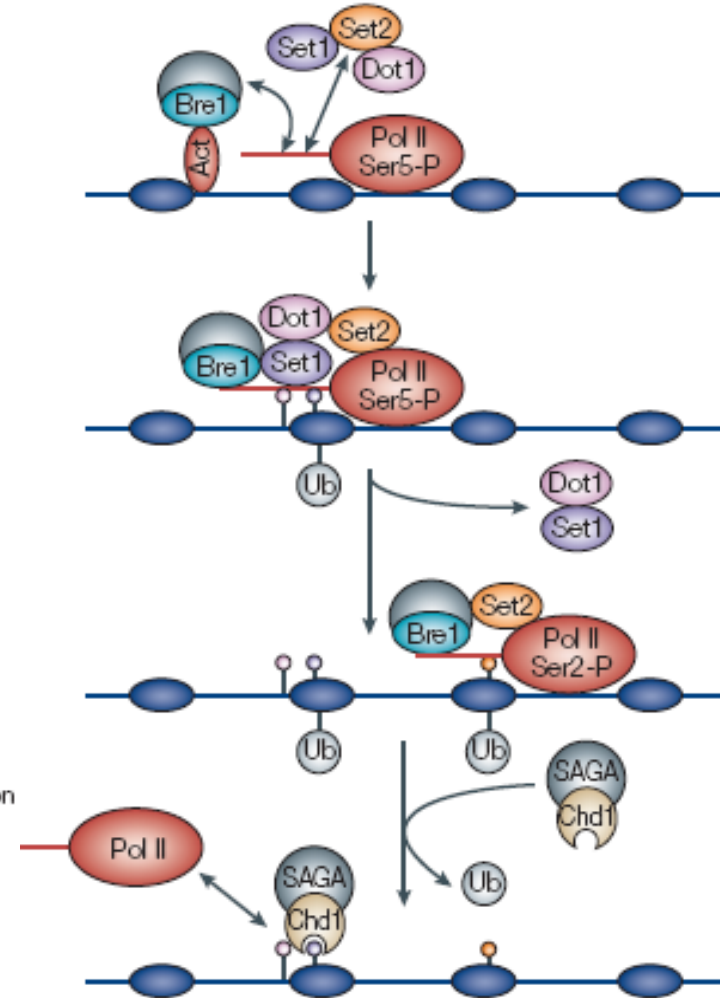
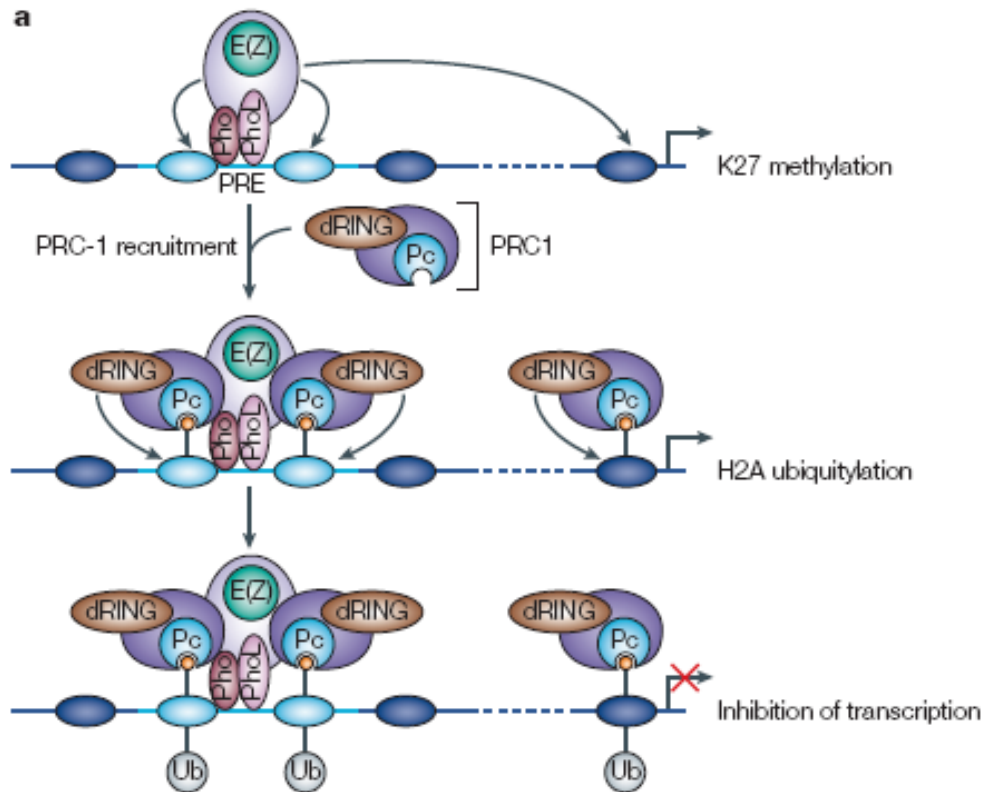


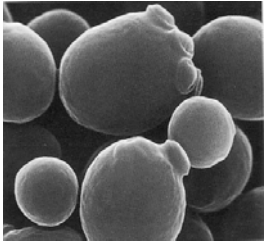
# Histone Methylation in Mating Type Switch



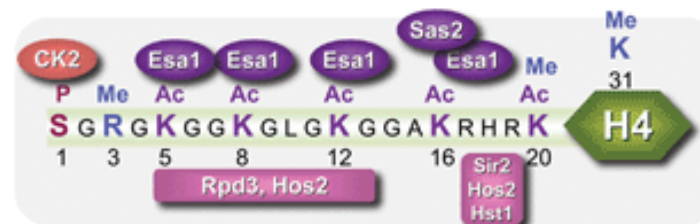
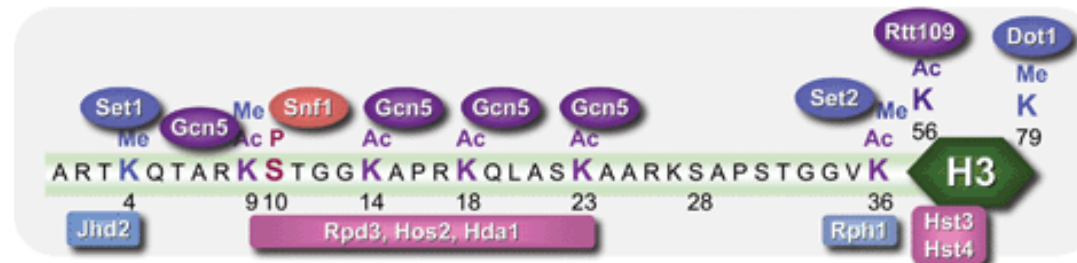


# Histone Methylation in Transcription

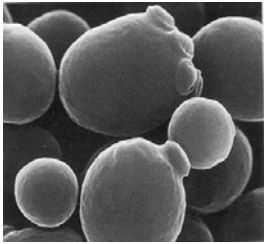




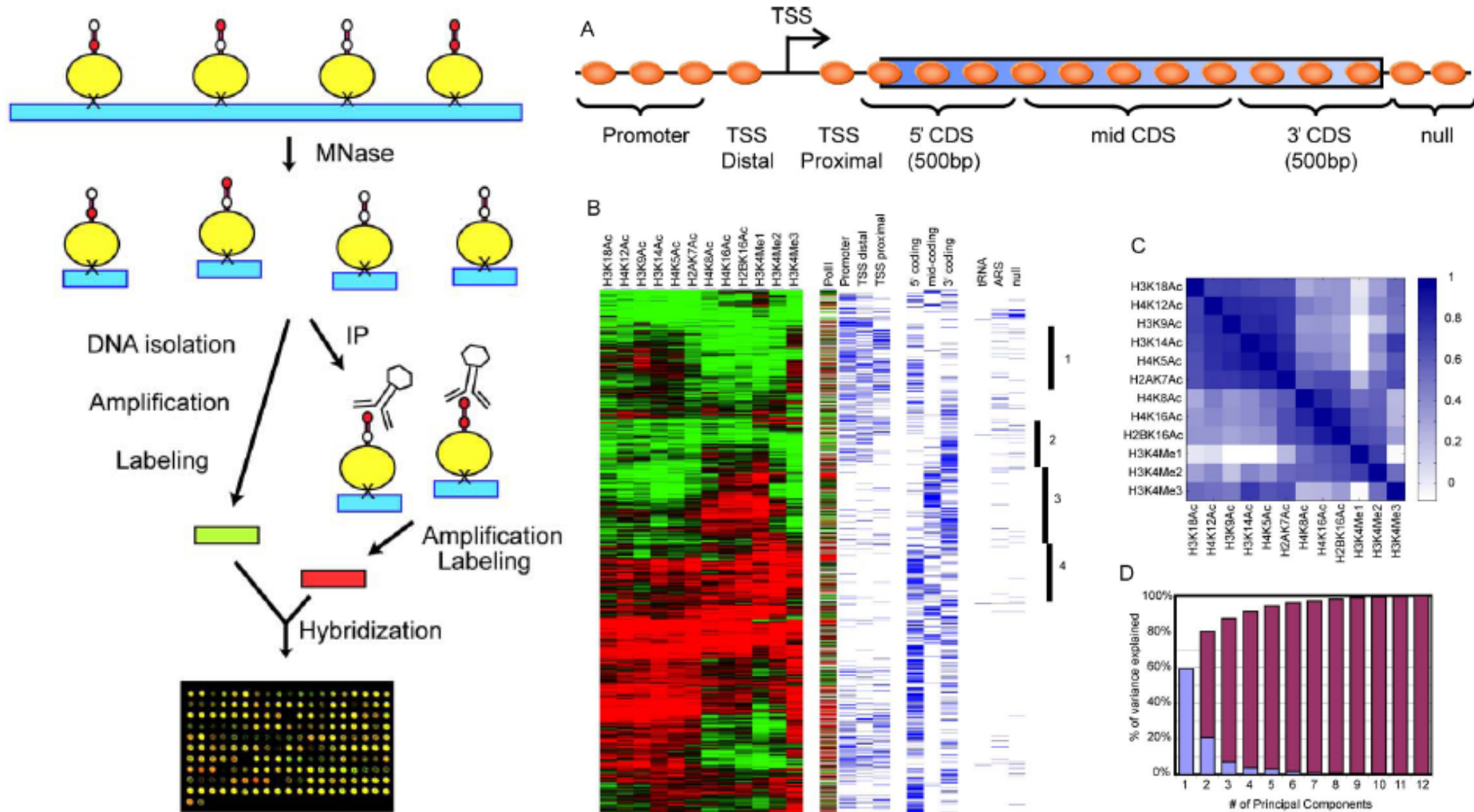
# The Histone Code

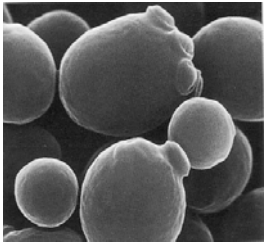




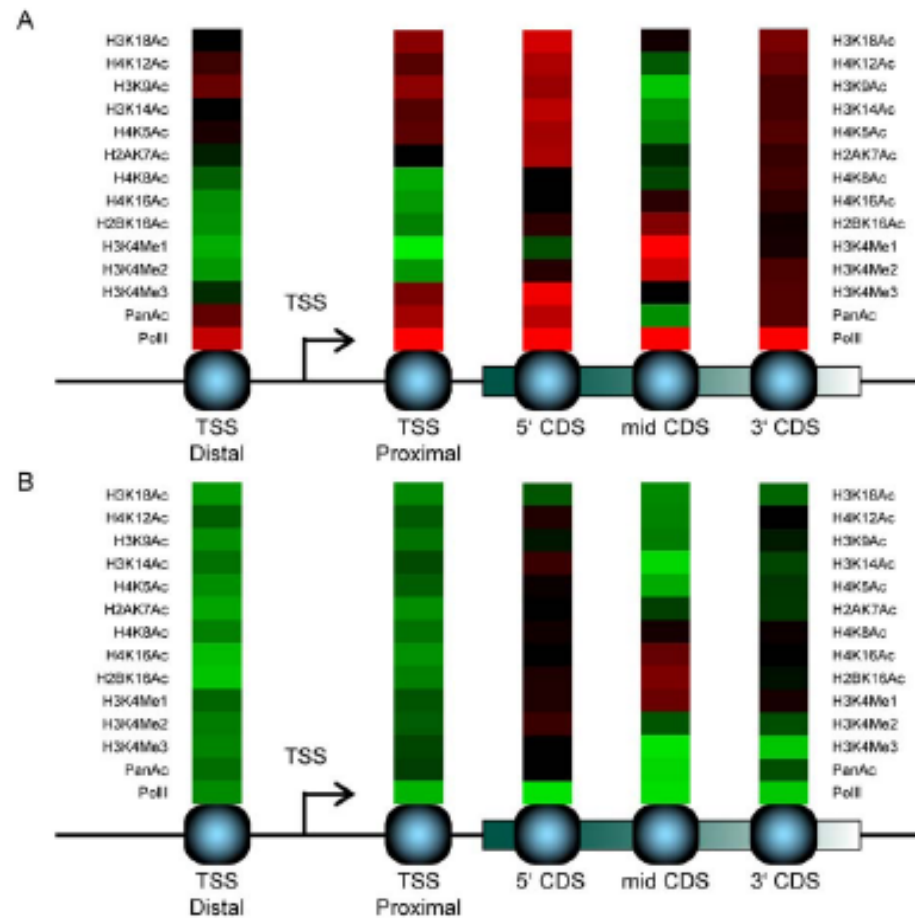


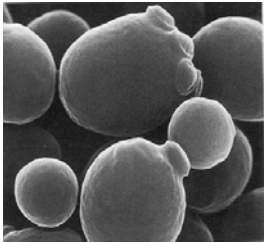
# Genomic Histone Modification Distribution



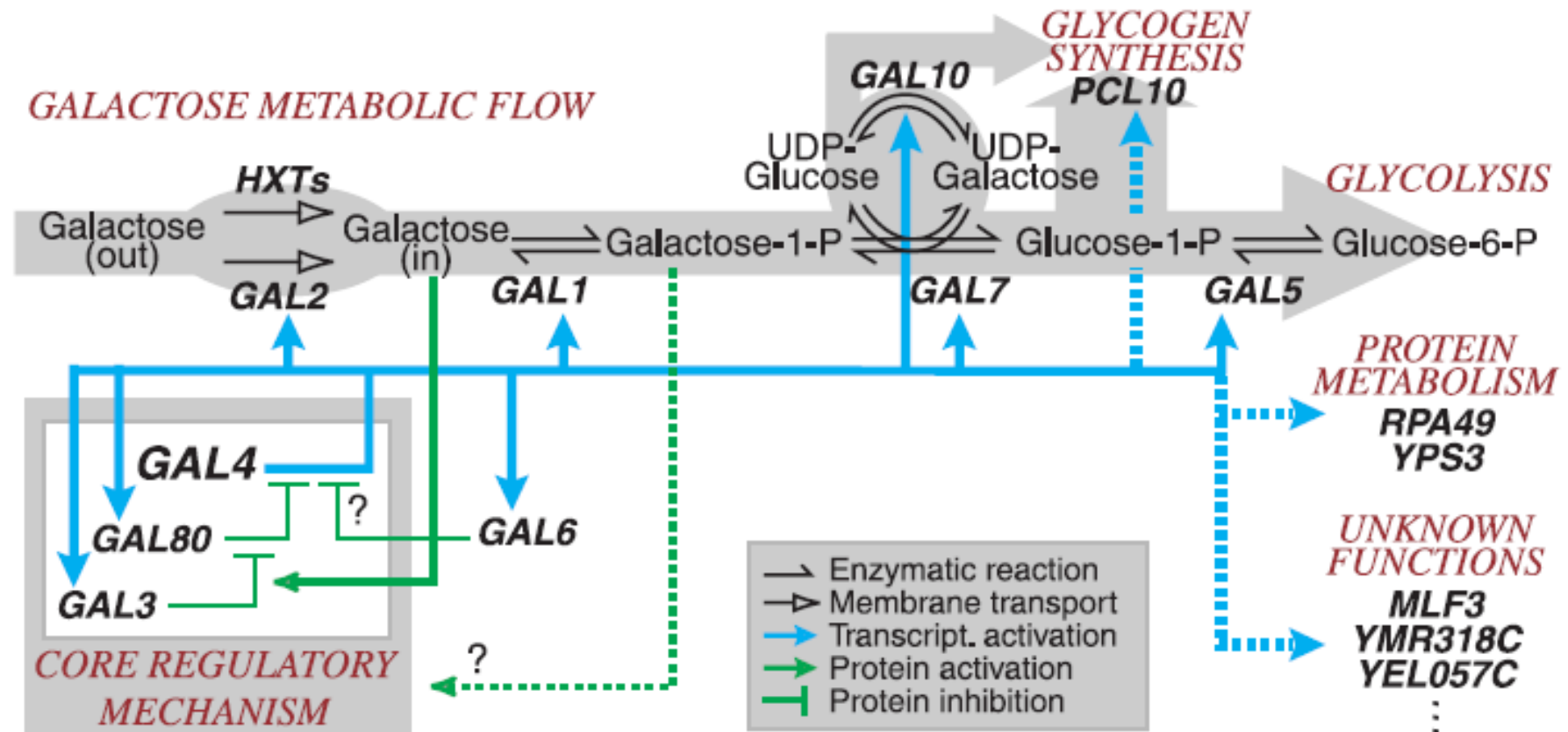


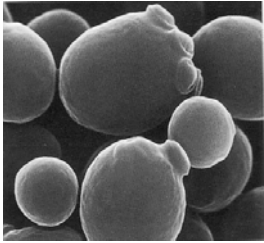
# RNA Polymerase Type-dependent Histone Modification Pattern



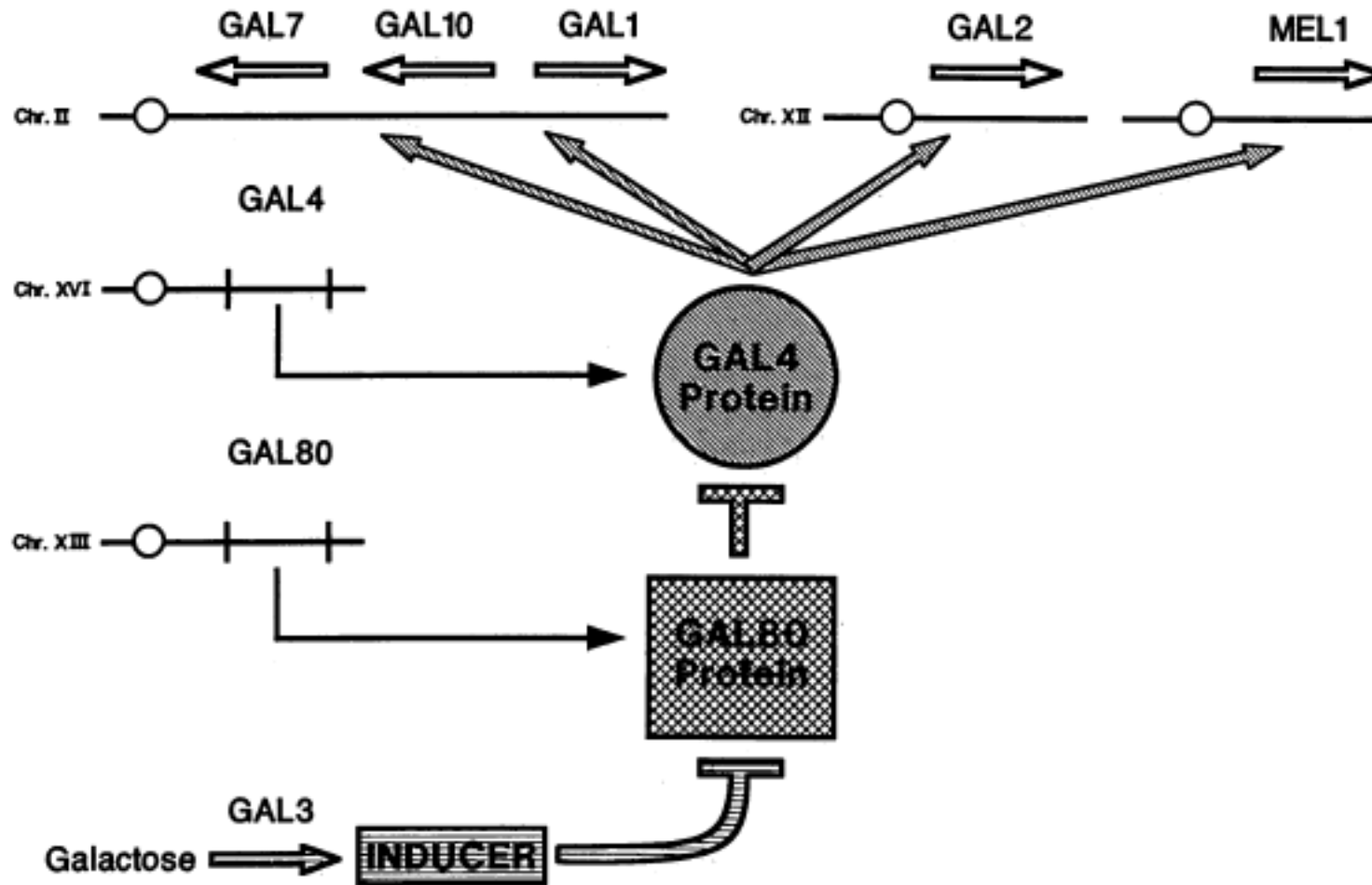


# Galactose Response Circuit



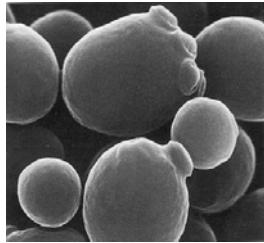


# Transcription in Galactose Response Circuit



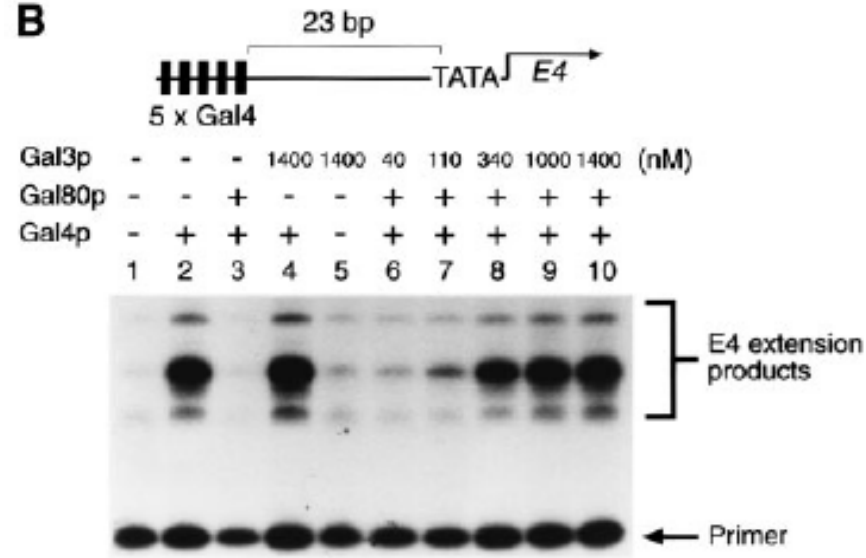


C G G A G G A C A C A G G A G G C  
19 20 18 10 16 9 13 11 20



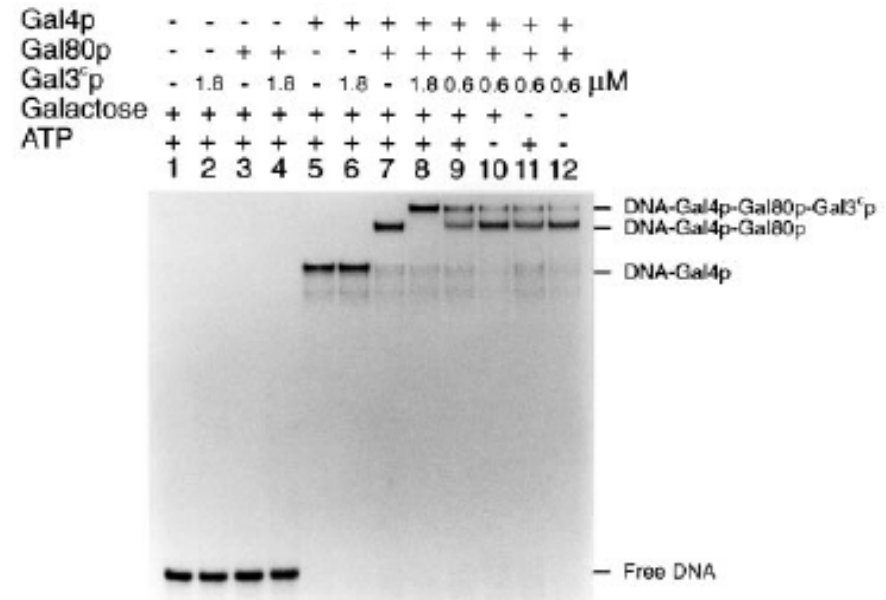
# Basic Mechanism in Gal3p-Gal80p-Gal4p Complex Function

**B**

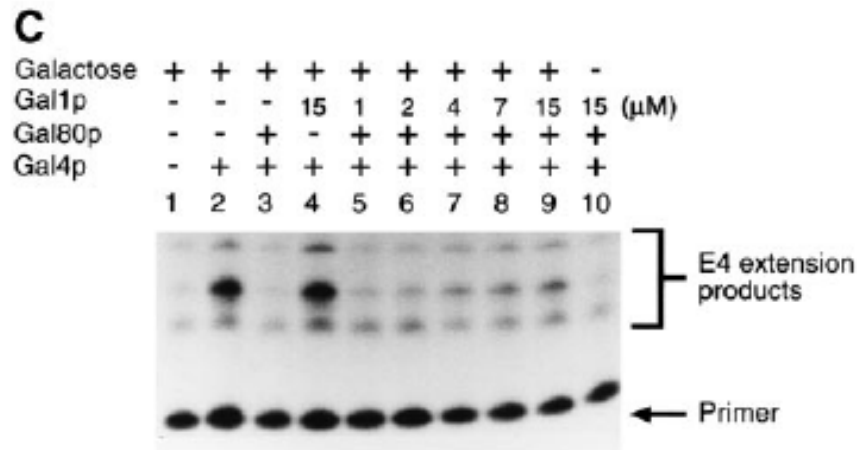


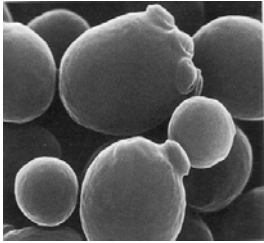
The EMBO Journal Vol.17 No.14 pp.4086-4091, 1998

The yeast galactose genetic switch is mediated by the formation of a Gal4p-Gal80p-Gal3p complex

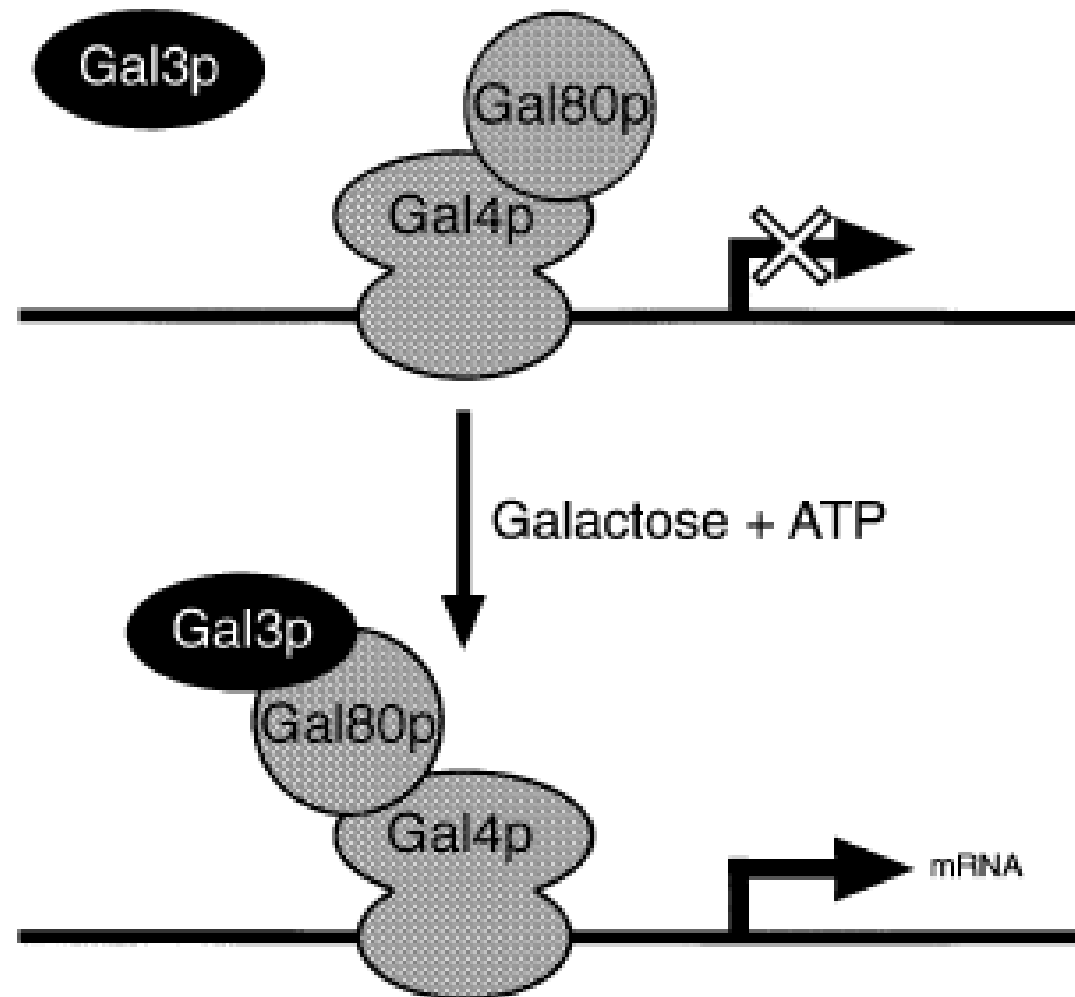


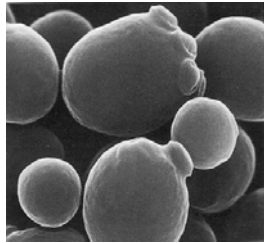
**C**



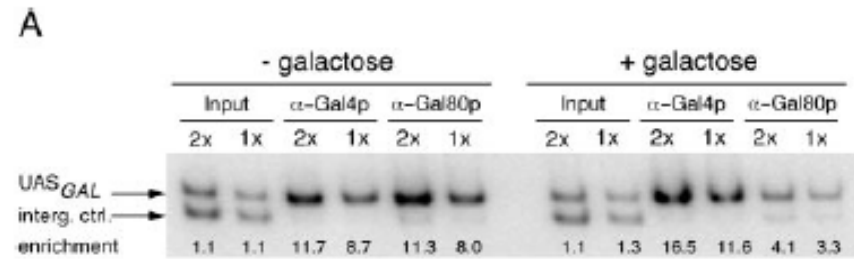
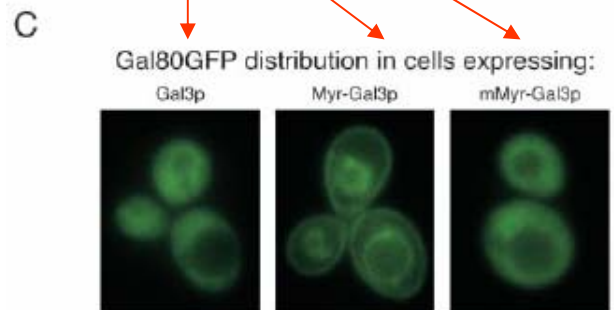
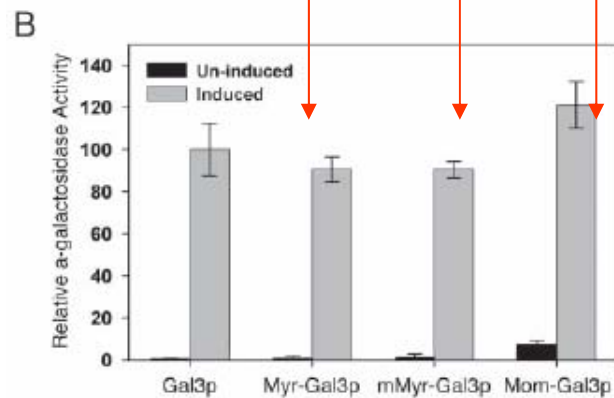
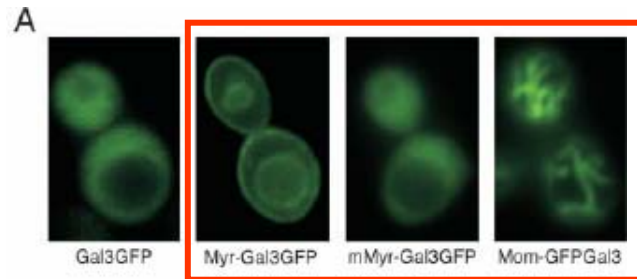


## First Model of Gal3p-Gal80p-Gal4p Complex Function (*in vitro*)

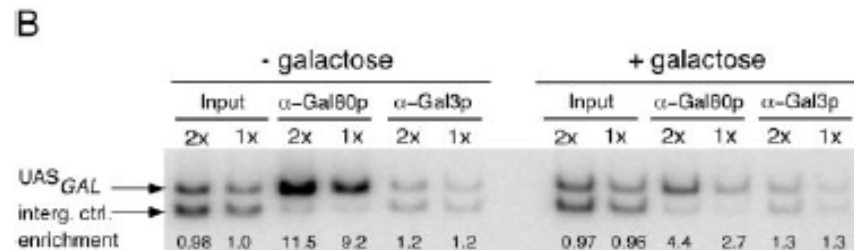




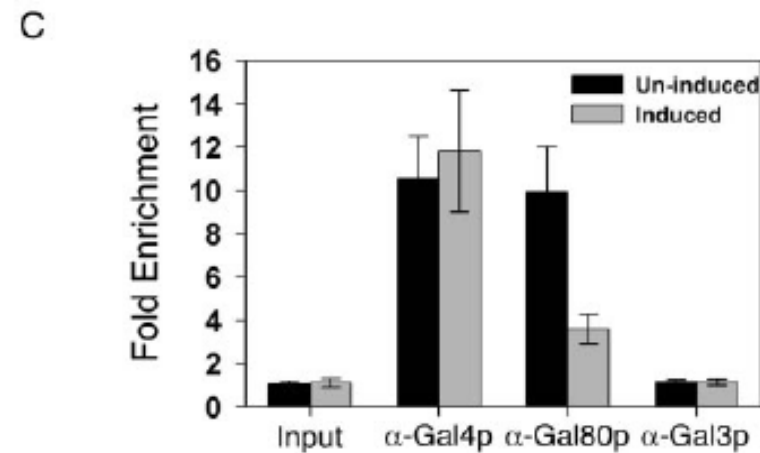
# Gal3p Functions in Cytoplasm to Sequester Shuttling Gal80p



ChIP

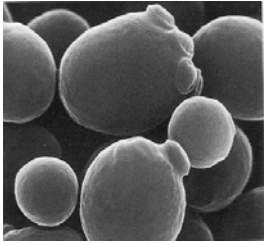


ChIP

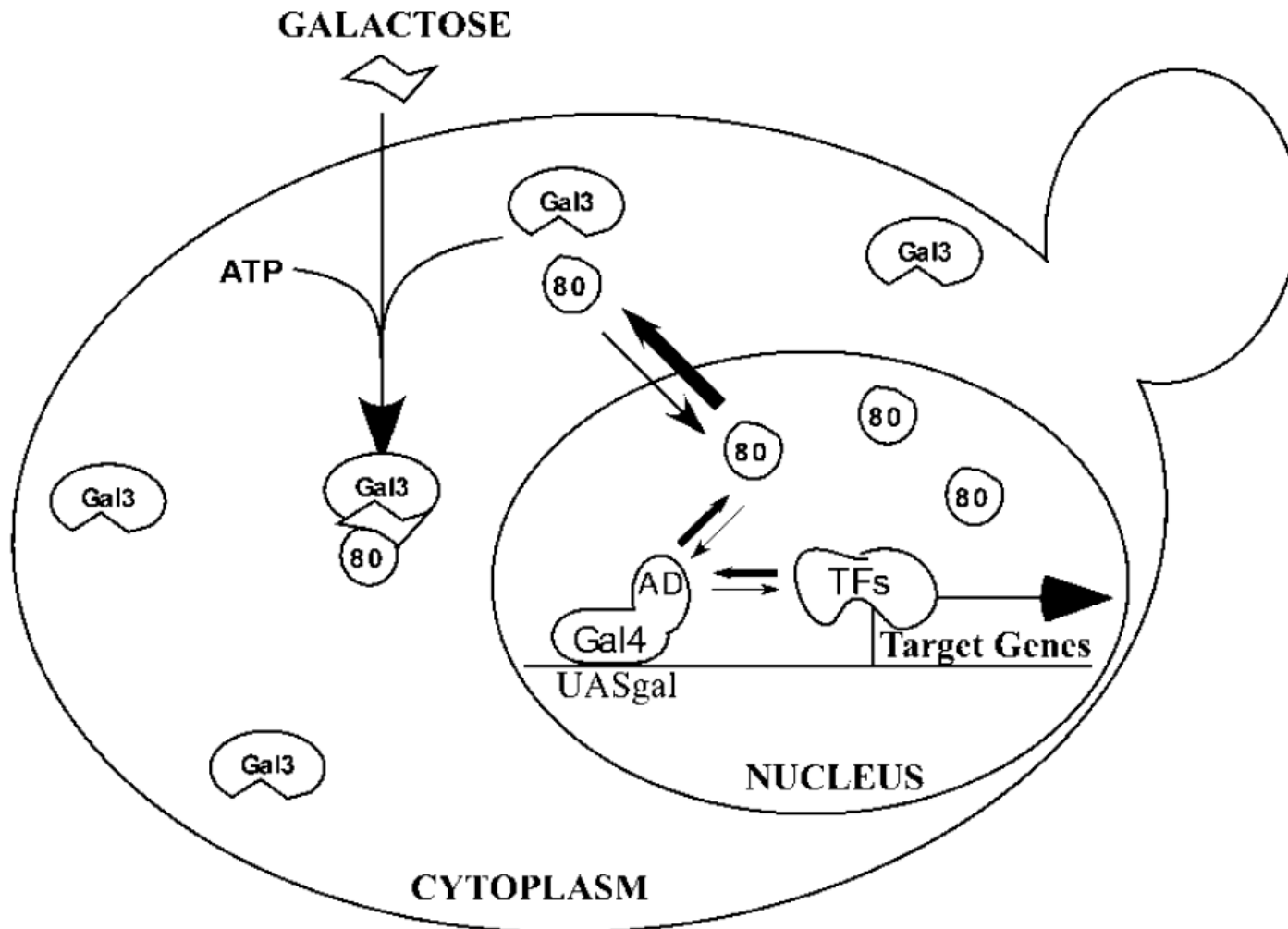


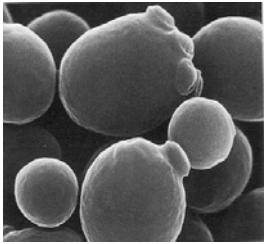
Peng and Hopper 2002



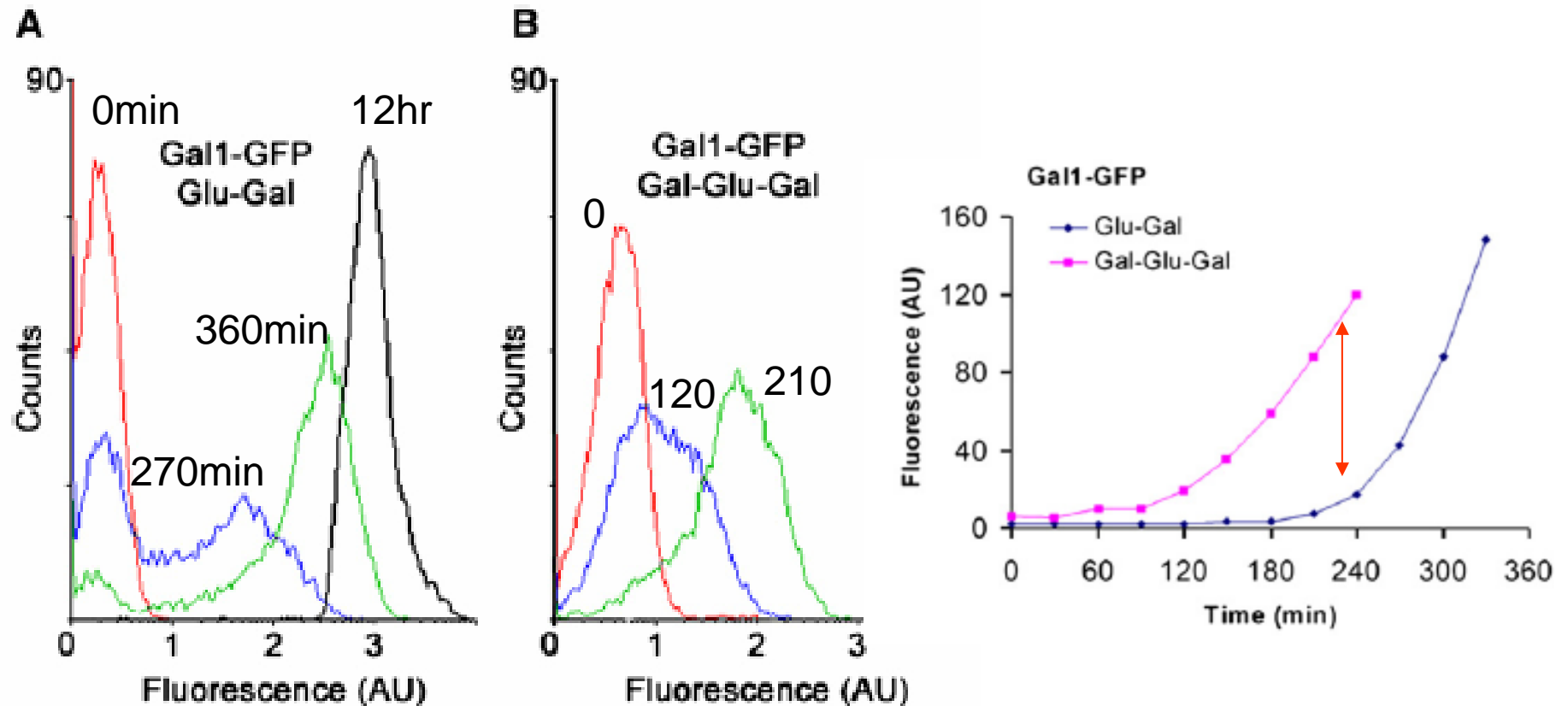


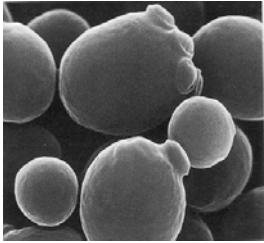
## Second Model of Gal3p-Gal80p-Gal4p Complex Function (*in vivo*)



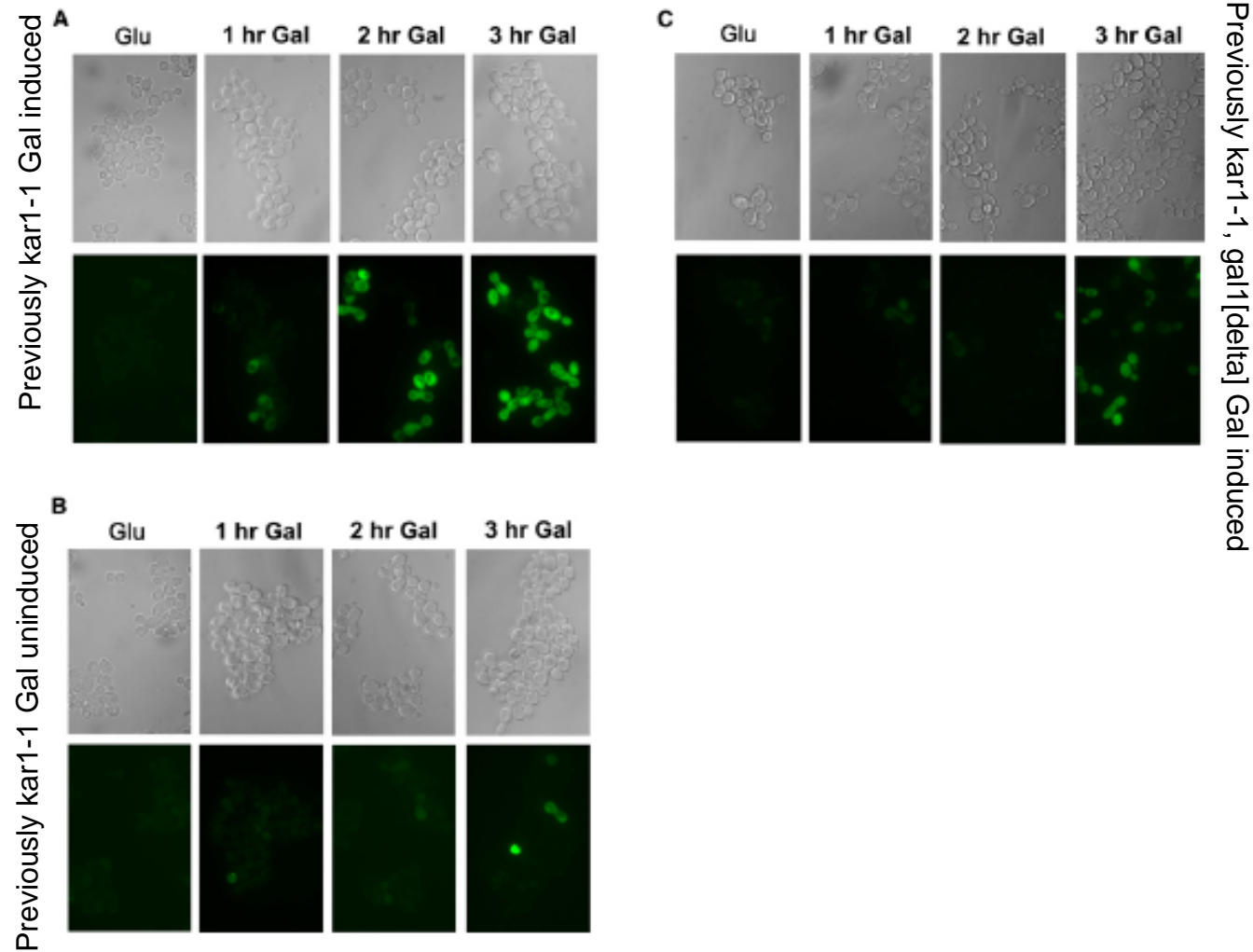


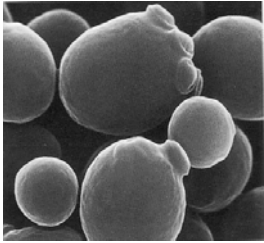
# Transcriptional Memory in Galactose Response Circuit



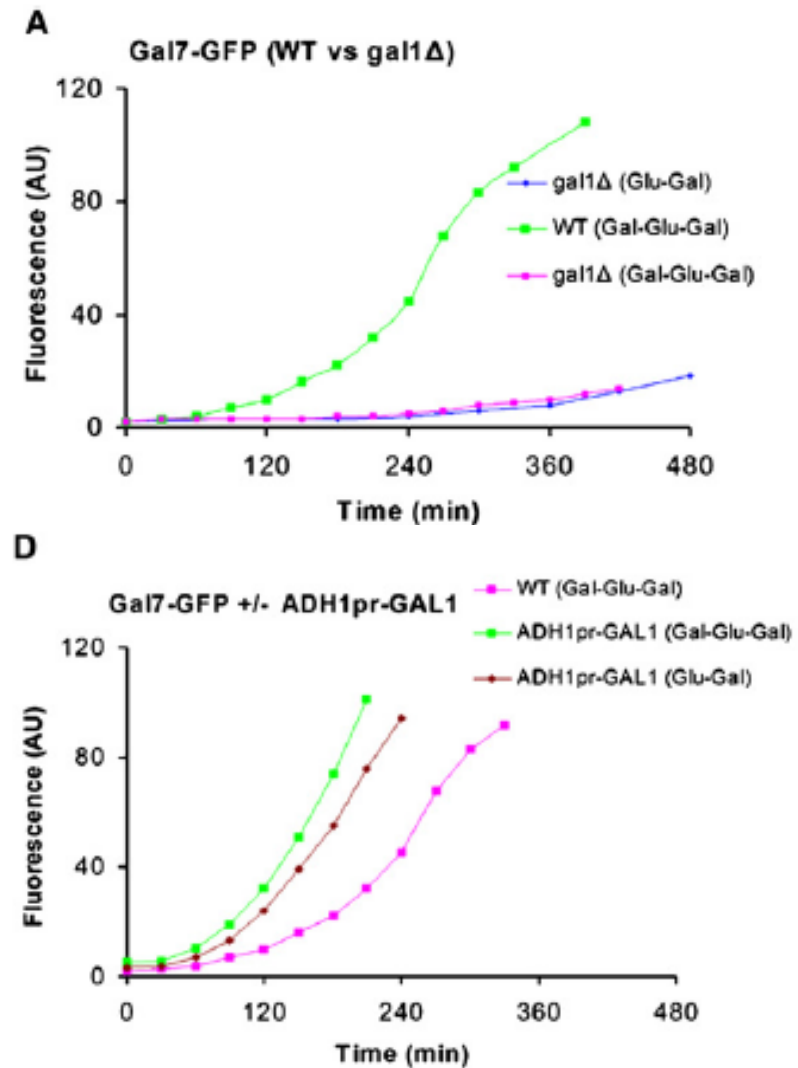
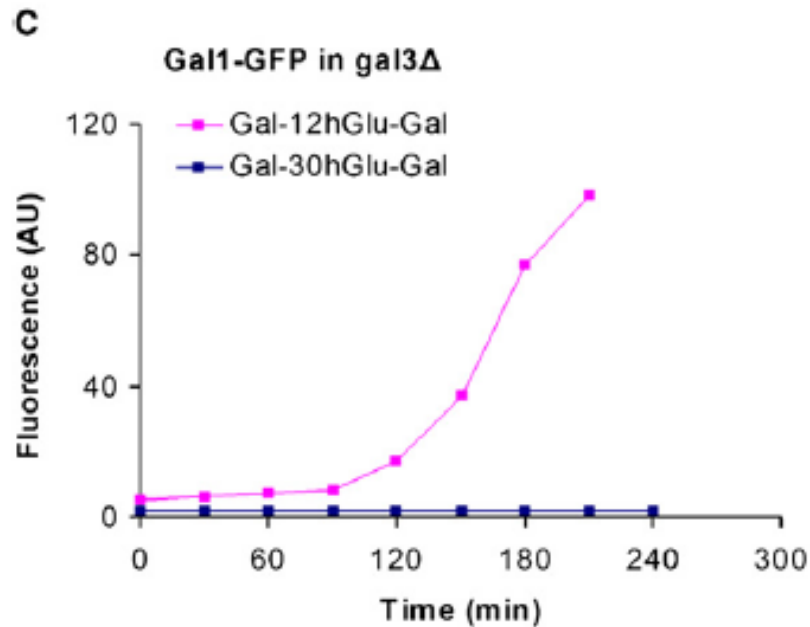


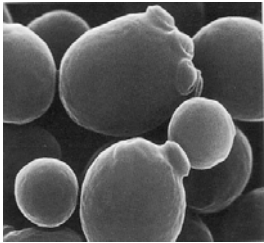
# Transcriptional Memory is Mediated by Cytoplasmic Gal1p



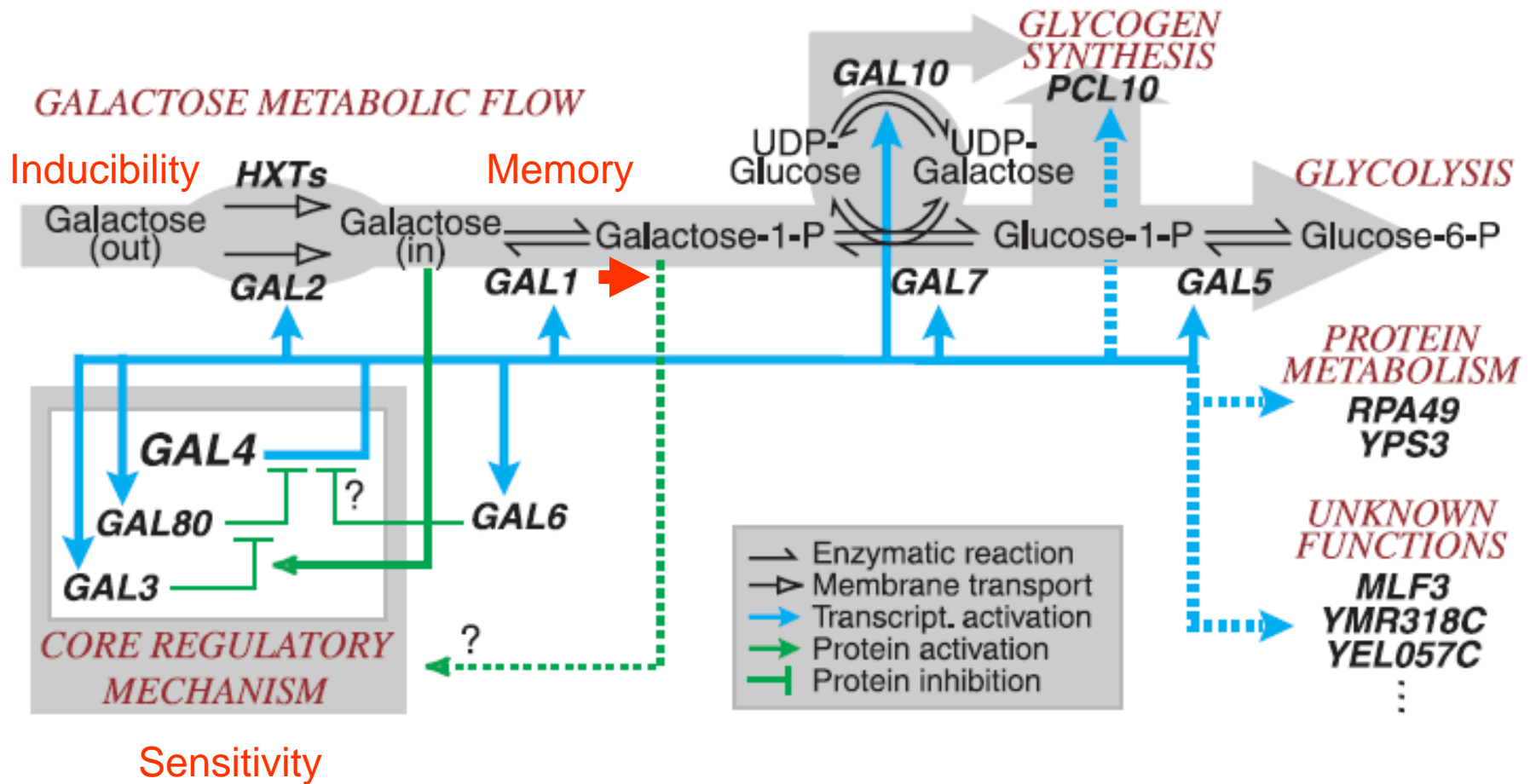


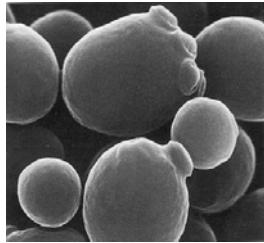
# Cytoplasmic Gal1p is necessary and sufficient for memory



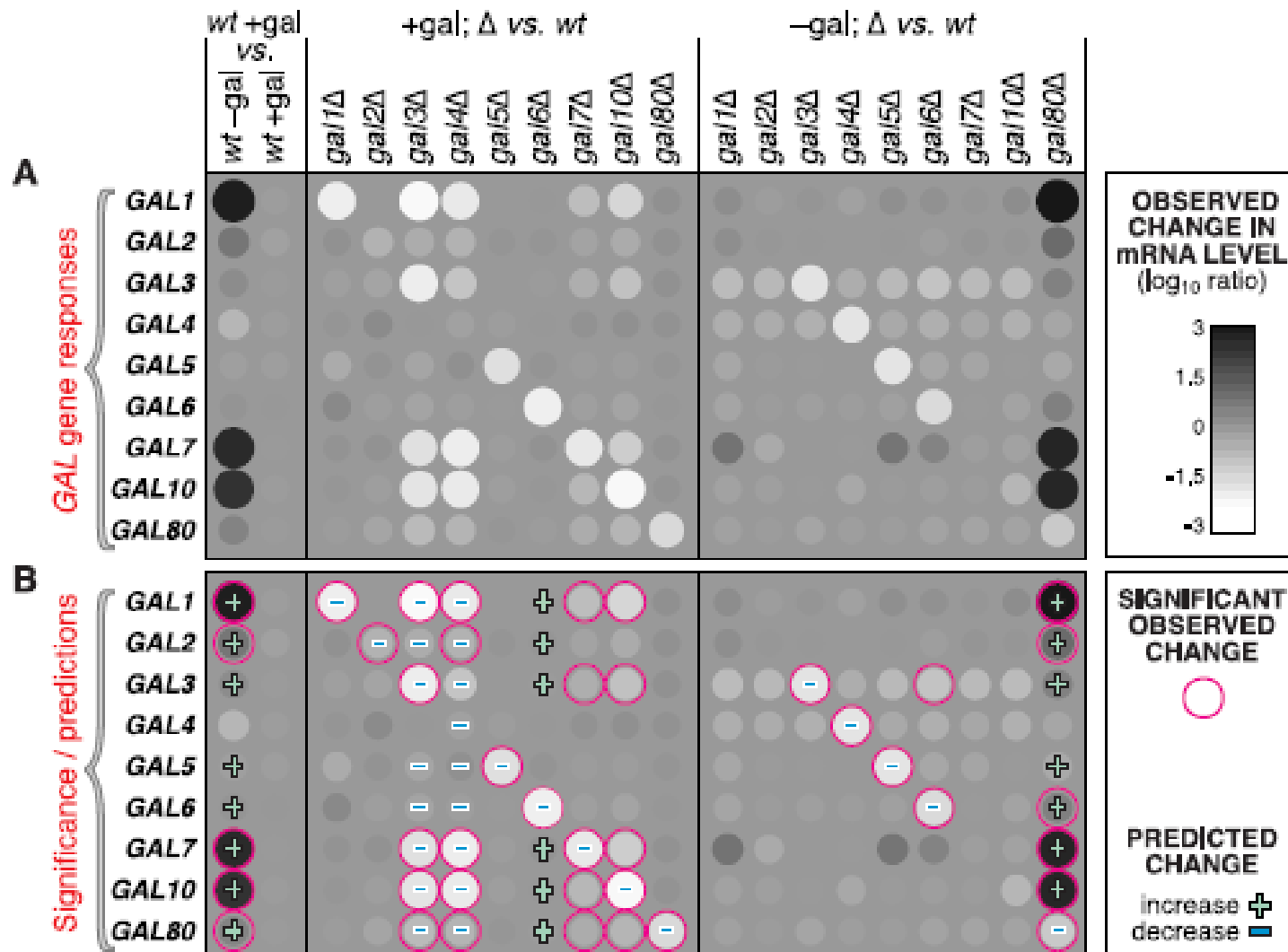


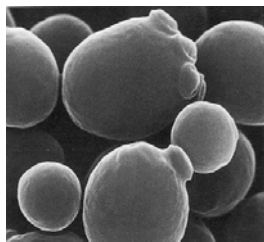
# Revisit the Circuit



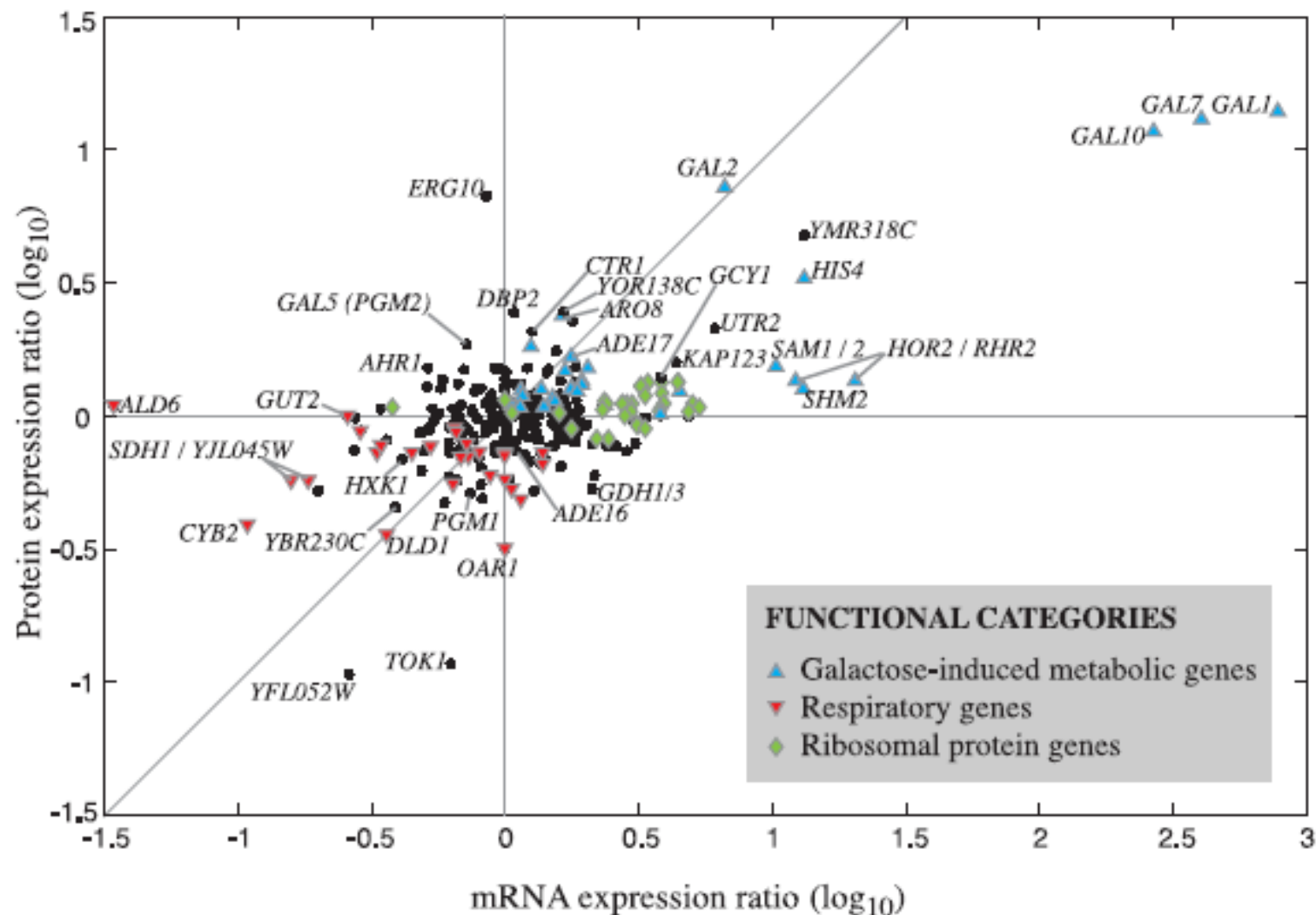


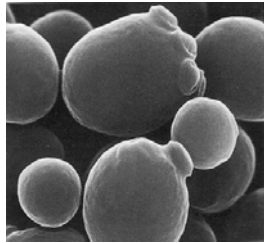
# Genomic mRNA Response to Circuit Perturbation



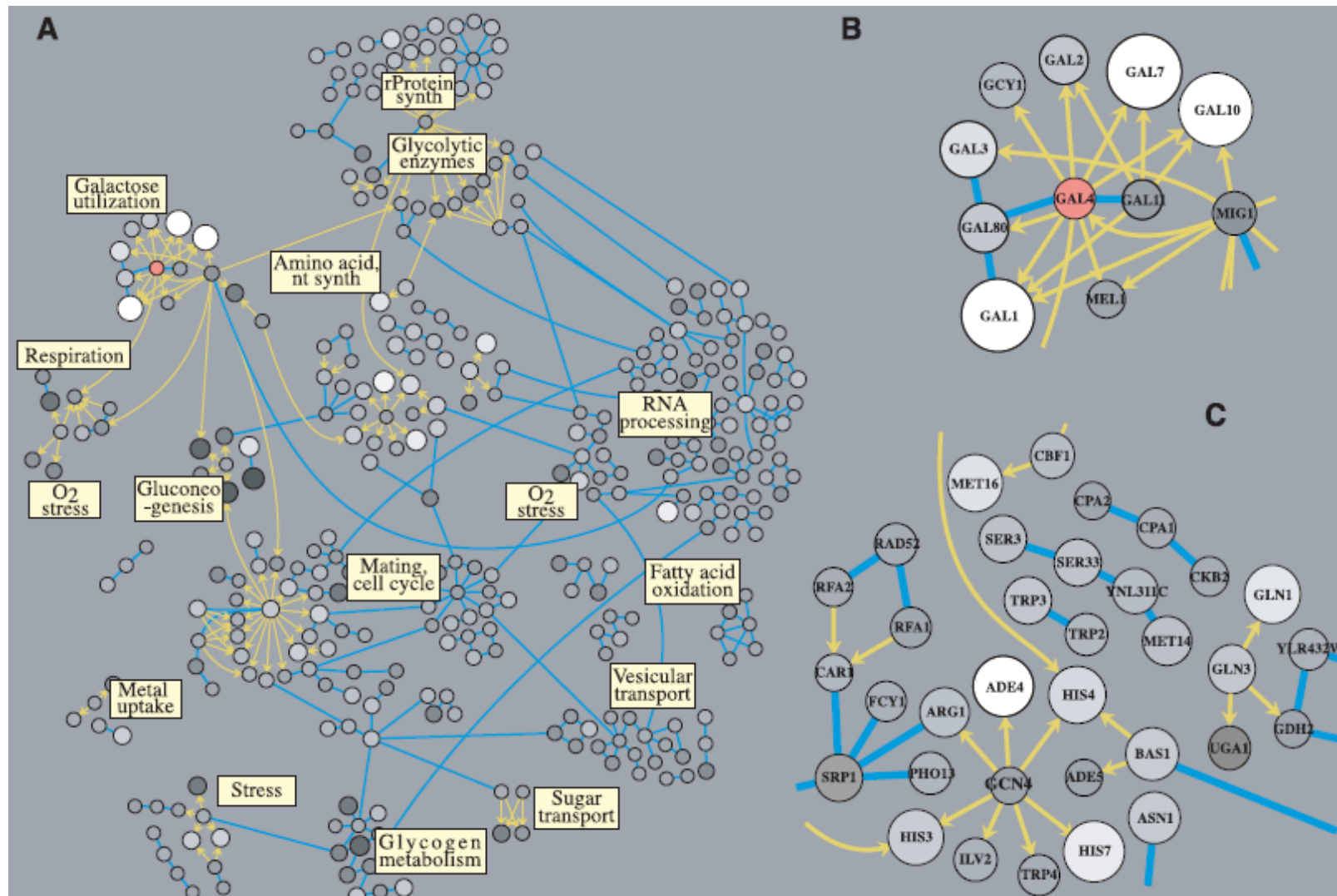


# Protein-mRNA Discrepancy in Systematic Response

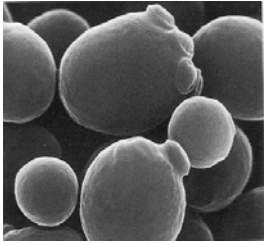




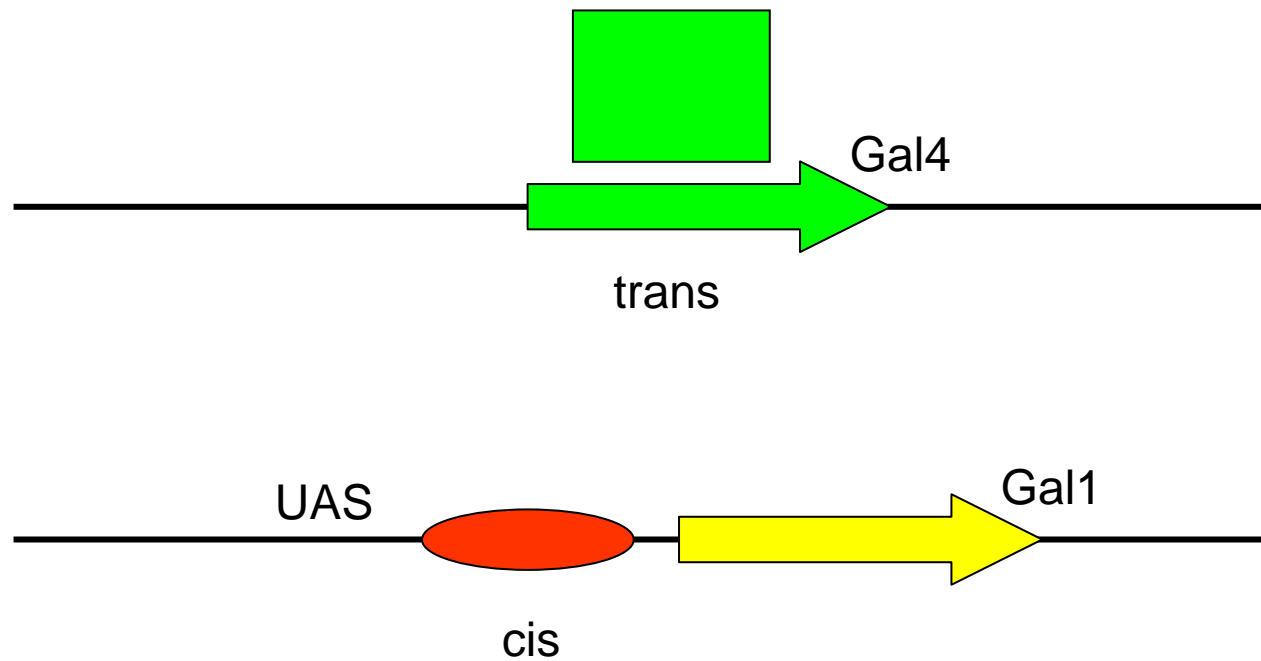
# Systematic Response: The Genome is a Network

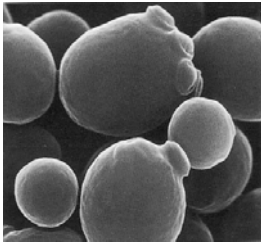






# Cis- and Trans-acting Elements

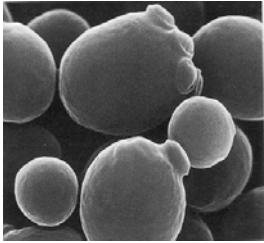




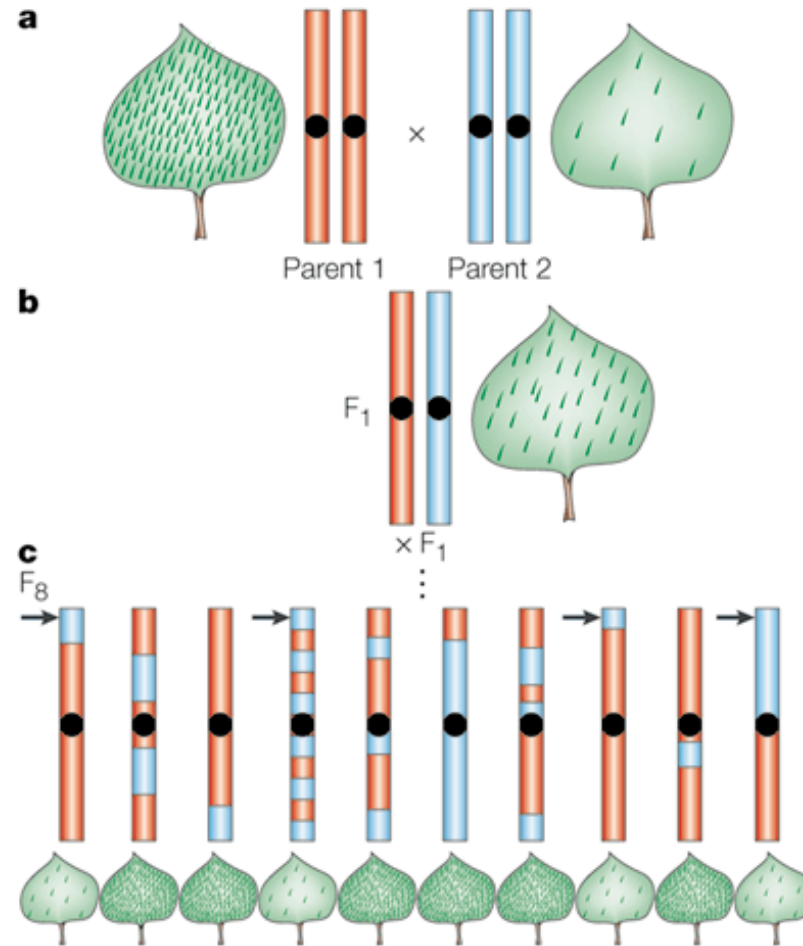
# Cis- and Trans- mechanisms in Network Architecture

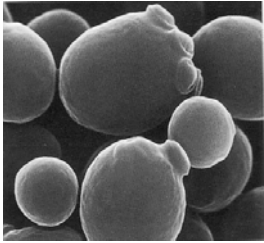
	Transcription factors	miRNAs
Pleiotropy		
Combinatorial and cooperative activity		
Accessibility		
Regulation		
Network motifs		

Example:  
feedforward loop



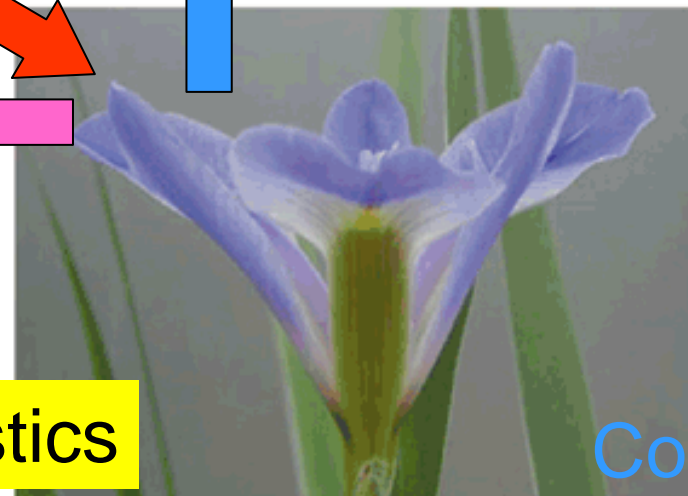
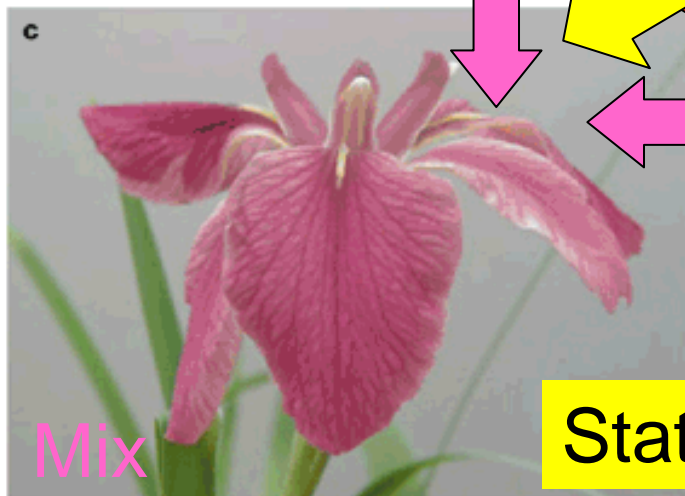
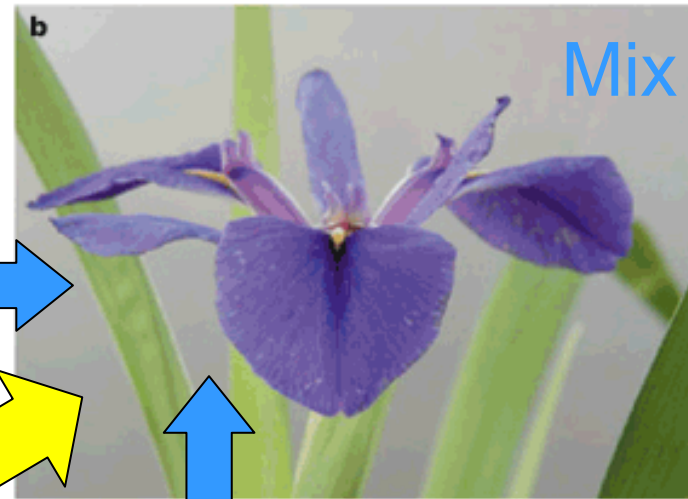
# Forward Genomics: Quantitative Trait Loci



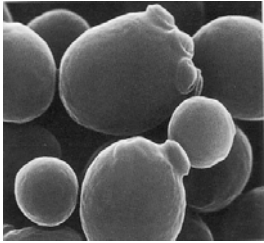


# Quantitative Trait Loci Principles

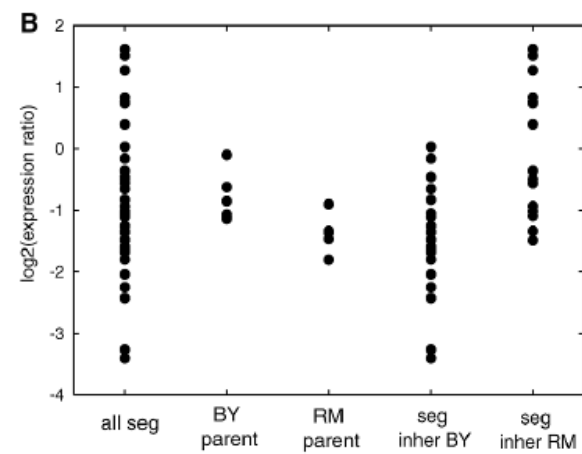
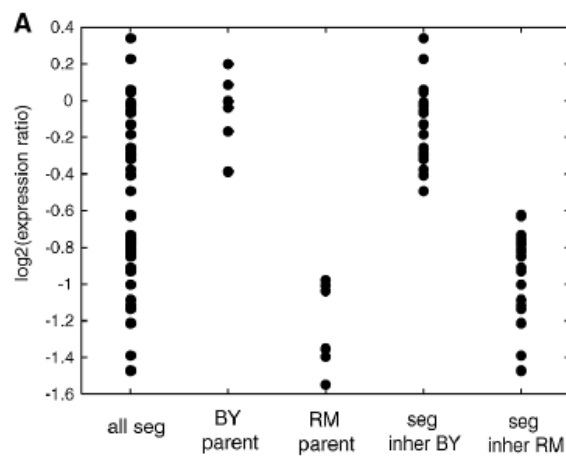
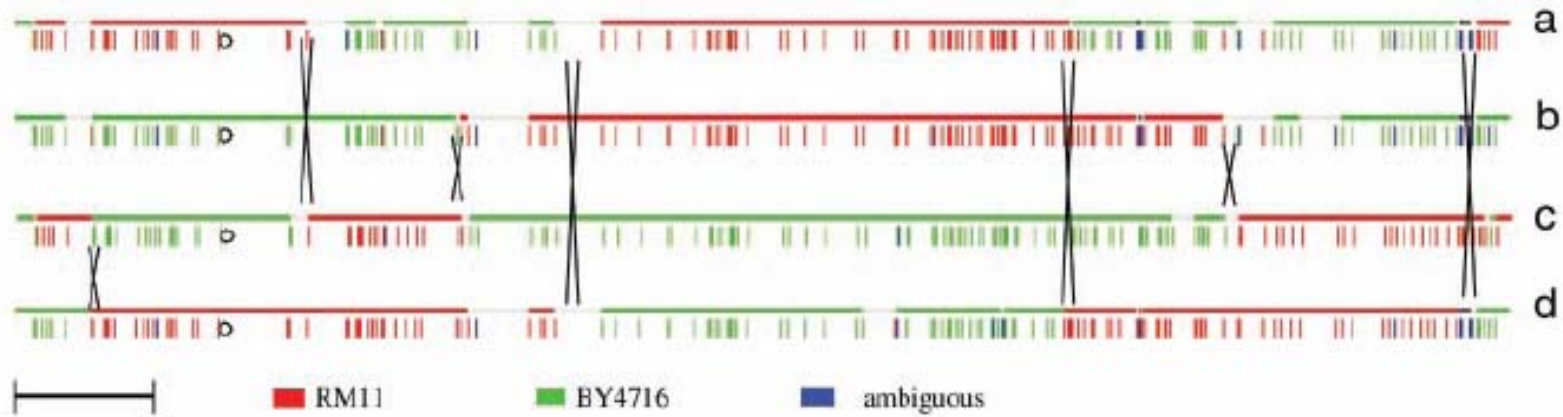
Contrast

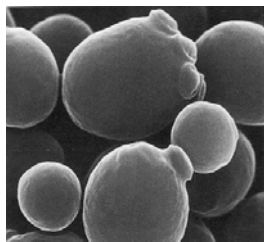


Statistics



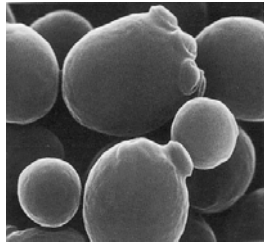
# QTL in Yeast Using Expression as a Phenotype



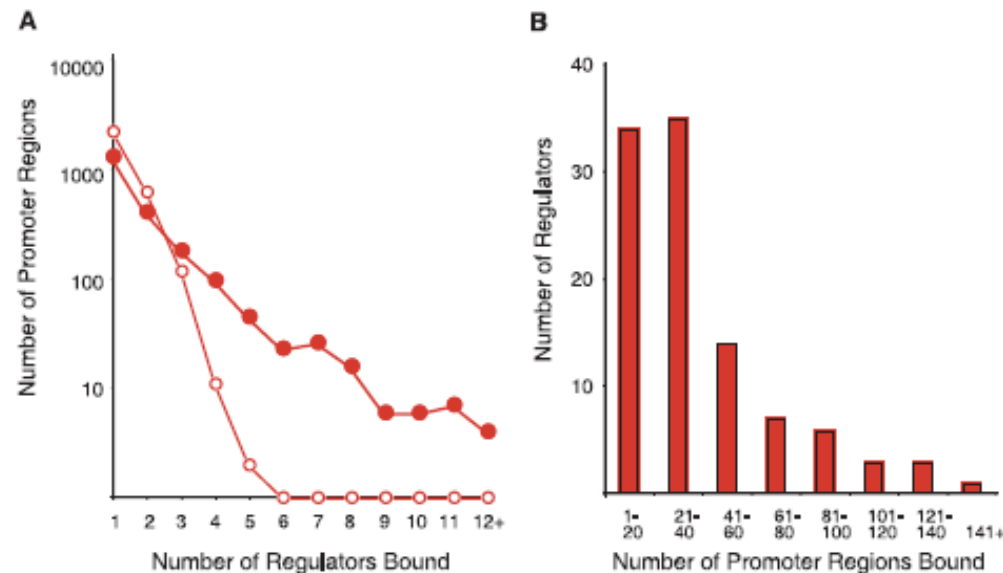
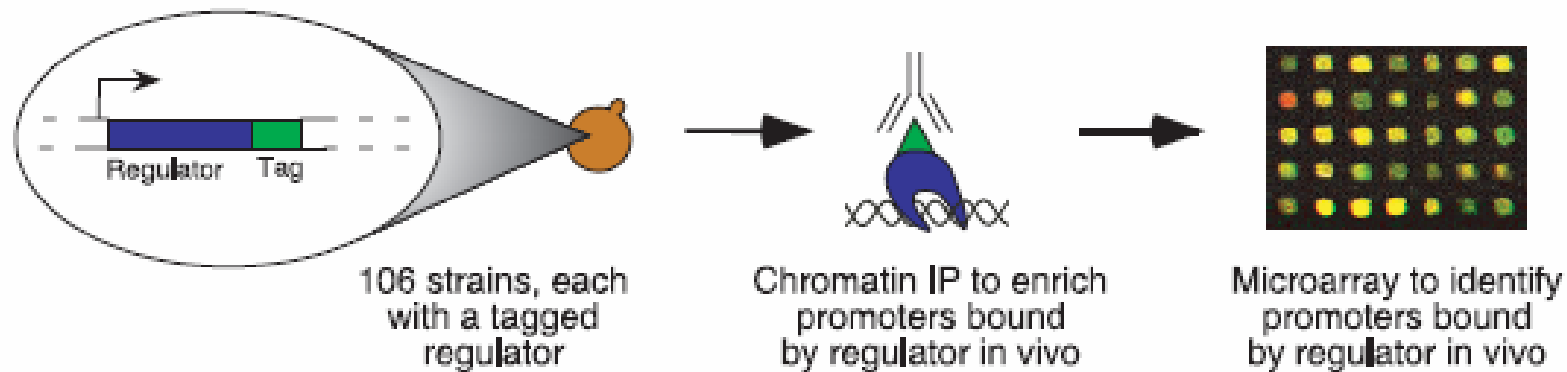


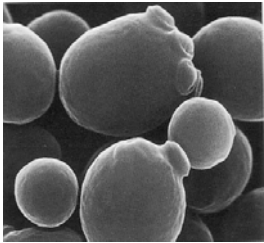
# Trans- acting elements in QTL

Group	Number of messages	Common function	Linkage bin	Putative regulator
1	18	Budding, daughter cell separation	II:550000	<i>CST13</i>
2	21	Leucine biosynthesis	III:90000	<i>LEU2</i>
3	28	Mating	III:190000	<i>MAT</i>
4	7	Uracil biosynthesis	V:110000	<i>URA3</i>
5	28	Heme, fatty acid metabolism	XII:670000	<i>HAP1</i>
6	16	Subtelomerically encoded helicases	XII:1030000	<i>SIR3</i>
7	94	Mitochondrial	XIV:490000	Unknown
8	19	Msn2/4-dependent induction in acid	XV:170000	Unknown



# Reverse Genomics: Direct Visualizing by ChIP-on-Chip



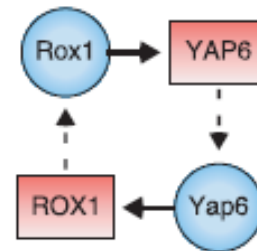


# Uncovered Network Motifs

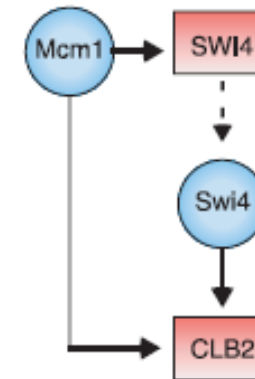
Autoregulation



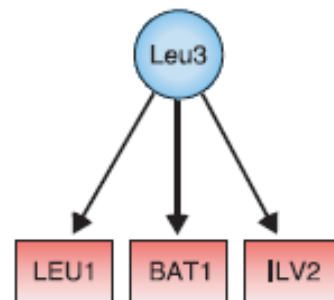
Multi-Component Loop



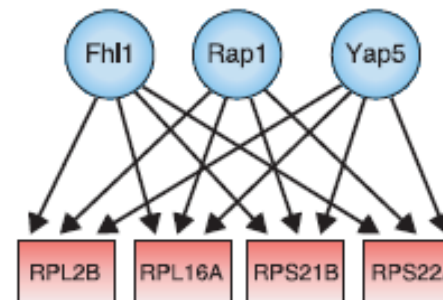
Feedforward Loop



Single Input Motif



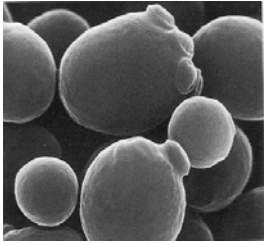
Multi-Input Motif



Regulator Chain







# Recovered Network in Cell Cycle

